(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
27 July 2006 (27.07.2006)

PCT

(10) International Publication Number
WO 2006/077441 A1

(51) International Patent Classification:
C07K 16/22 (2006.01) *C07K 14/48* (2006.01)

(21) International Application Number: PCT/GB2006/000238

(22) International Filing Date: 24 January 2006 (24.01.2006)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
60/645,587 24 January 2005 (24.01.2005) US

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(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KN, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, LY, MA, MD, MG, MK, MN, MW, MX, MZ, NA, NG, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SM, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW.

(84) Designated States *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IS, IT, LT, LU, LV, MC, NL, PL, PT, RO, SE, SI, SK, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Declaration under Rule 4.17:
— *of inventorship (Rule 4.17(iv))*

Published:
— *with international search report*

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

(54) **Title:** SPECIFIC BINDING MEMBERS FOR NGF

(57) **Abstract:** Specific binding members for Nerve Growth Factor (NGF), in particular anti-NGF antibody molecules, especially human antibody molecules, and especially those that neutralise NGF activity. Methods for using anti-NGF antibody molecules in diagnosis or treatment of NGF related disorders, including pain, asthma, chronic obstructive pulmonary disease, pulmonary fibrosis, other diseases of airway inflammation, diabetic neuropathy, cardiac arrhythmias, HIV, arthritis, psoriasis and cancer.

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SPECIFIC BINDING MEMBERS FOR NGF

INCORPORATION BY REFERENCE OF MATERIAL SUBMITTED ON A DISKETTE

A diskette copy of the Sequence Listing of Sequences 1 to 537 which is saved as sequence listing.txt and is 253 KB, and is submitted herewith and is incorporated by reference herein in its entirety for all purposes.

The present invention relates to specific binding members, in particular anti-NGF antibody molecules, especially human antibody molecules, and especially those that neutralise NGF (Nerve Growth Factor) activity. It further relates to methods for using anti-NGF antibody molecules in diagnosis or treatment of NGF related disorders, including pain, asthma, chronic obstructive pulmonary disease, pulmonary fibrosis, other diseases of airway inflammation, diabetic neuropathy, cardiac arrhythmias, HIV, arthritis, psoriasis and cancer.

The present invention provides antibody molecules of particular value in binding and neutralising NGF, and thus of use in any of a variety of therapeutic treatments, as indicated by the experimentation contained herein and further by the supporting technical literature.

Nerve growth factor (β-NGF, commonly known as NGF) plays a well-known pivotal role in the development of the nervous system. In the adult, however, NGF plays a more restricted role, where it promotes the health and survival of a subset of central and peripheral neurons (Huang & Reichardt, 2001). NGF also contributes to the modulation of the functional characteristics of these neurons. As part of this latter process, NGF exerts tonic control over the sensitivity, or excitability, of nociceptors (Priestley et al., 2002; Bennett, 2001). These peripheral neurons sense and transmit to the central nervous system the various noxious stimuli that ultimately give rise to perceptions of pain (nociception). Thus, agents that reduce levels of NGF may possess utility as analgesic therapeutics.

The societal cost of inadequately treated pain further supports the potential utility of analgesics based on anti-NGF activity. That is, despite the existence and widespread use of numerous pain medications, a clear need exists for new analgesics. Pain is one of the most common symptoms for which medical assistance is sought and is the primary complaint of half of all patients visiting a physician. The high cost of pain to society is well documented. In the U.S., for example, chronic pain afflicts some 34 million Americans. Pain results in 50 million workdays lost each year. Direct medical costs attributed to back pain, arthritic pain, and migraine amount to $40 billion annually alone. The total prescription pain medication market is approximately $15 billion per year (Pleuvry & Pleuvry).

As these statistics imply, a substantial percentage of pain sufferers fail to receive adequate pain relief. As a consequence, a large medical need remains for safe and effective analgesics with novel mechanisms of action (Pleuvry & Pleuvry).

Therapeutic agents that reduce the tissue levels or inhibit the effects of secreted NGF have the potential to be just such novel analgesics. Subcutaneous injections of NGF itself produce pain in humans and animals. Thus, injected NGF causes a rapid thermal hyperalgesia, followed by delayed thermal hyperalgesia and mechanical allodynia (Petty et al., 1994; McArthur et al., 2000). Endogenously secreted NGF is similarly pro-nociceptive. Tissue-injury-induced release of NGF and its subsequent action in the periphery plays a major role in the induction of thermal hyperalgesia through the process of 'peripheral sensitization' (Mendell & Arvanian, 2002). Tissue injury promotes the release of pro-nociceptive and pro-inflammatory cytokines, which, in turn, induce the release of NGF from keratinocytes and fibroblasts. This released NGF acts directly on nociceptors to induce painful or nociceptive states within minutes of the noxious insult. This NGF also acts indirectly to induce and maintain nociceptive/pain states. It triggers mast cell

degranulation, releasing pro-nociceptive agents such as histamine and serotonin and, importantly, more NGF, and can also stimulate sympathetic nerve terminals to release pro-nociceptive neurotransmitters, such as noradrenaline (Ma & Woolf, 1997).

Tissue levels of NGF are elevated in CFA- and carrageenan-injected animals (Ma & Woolf, 1997; Amann & Schuligoi, 2000). Moreover, increased levels of NGF have been documented in patients suffering from rheumatoid arthritis (Aloe & Tuveri, 1997) or cystitis (Lowe et al., 1997). In rodents, peripheral nerve injury increases the expression of NGF mRNA in macrophages, fibroblasts, and Schwann cells (Heumann et al., 1987). Over-expression of NGF in transgenic mice results in enhanced neuropathic pain behavior following nerve injury above that of wild-type mice (Ramer et al., 1998). Over hours and days, elevated NGF levels play a role in promoting 'central sensitization' - the enhancement of neurotransmission at synapses in the nociceptive pathways of the spinal cord. Central sensitization results in persistent and chronic hyperalgesia and allodynia. This process is thought to involve internalization of complexes of NGF and its high affinity receptor, trkA (tyrosine receptor kinase A). Retrograde transport of these complexes to nociceptor cell bodies in the dorsal root ganglia (DRG) potentiates secretion of nociceptive neuropeptides (e.g., substance P, CGRP), PKC activation, and NMDA receptor activation in the dorsal horn of the spinal cord (Sah et al., 2003) - all processes that promote the sensitization of the nociceptive pathways. NGF also plays a role in the up-regulation and re-distribution of voltage-dependent and ligand-gated ion channels, including sodium channel subtypes and the capsaicin receptor, VR1 (Mamet et al., 1999; Fjell et al., 1999; Priestley et al., 2002). The altered activities and/or expression of transmitters, receptors, and ion channels underlie the increased sensitivity and excitability of nociceptors associated with neuropathic pain states.

NGF can also promote the sprouting of sympathetic neurons and the formation of aberrant innervation of nociceptive neurons. This

innervation is thought to contribute to the induction and maintenance of chronic nociceptive/pain states, such as sympathetically maintained pain, or complex regional pain syndrome (Ramer et al., 1999).

NGF-induced nociception/pain is mediated by the high affinity NGF receptor, trkA (tyrosine receptor kinase A) (Sah, et al., 2003). About 40 - 45% of nociceptor cell bodies in DRGs express trkA. These are the cell bodies of the small diameter fibers, or C-fibers, that also express the secreted pro-nociceptive peptides, substance P and CGRP. These fibers terminate in laminae I and II of the dorsal horn, where they transfer to the central nervous system the noxious stimuli sensed by peripheral nociceptors. Mutations or deletions in the trkA gene produce a phenotype characterized by loss of pain sensation both in humans (Indo, 2002) and in trkA knock-out mice (de Castro et al., 1998). Significantly, the expression of trkA is up-regulated in animals subjected to models of arthritic (Pozza et al., 2000) or cystitic pain (Qiao & Vizzard, 2002), or the inflammatory pain induced by injection of complete Freund's adjuvant (CFA) or carrageenan into the paw (Cho et al., 1996).

NGF also binds to the p75 neurotrophin receptor. The role of the p75 receptor is dependent on its cellular environment and the presence of other receptors with which it is believed to play an accessory or co-receptor function. Interaction between the trkA and p75 receptors results in the formation of high affinity binding sites for NGF. The importance of such receptor interactions in NGF-mediated pain signalling is not clear, but recent studies have implicated the p75 receptor in cellular processes that may be relevant (Zhang & Nicol, 2004). However, whilst p75 receptor knockout mice display elevated thresholds to noxious stimuli, they remain responsive to the hyperalgesic effects of NGF, suggesting that trkA receptors alone are sufficient to mediate these effects (Bergmann *et al.,* 1998).

The evidence cited above indicates that NGF-mediated processes are responsible for the induction of acute pain, short-term pain,

persistent nociceptive pain, and persistent or chronic neuropathic pain. Thus, anti-NGF agents are indicated as having utility as effective analgesics for treating sufferers of any or all of these various pain states.

One such anti-NGF agent is trkA-Fc, which acts as a decoy or scavenger to bind up, and thereby inactivate, endogenous NGF. TrkA-Fc is a fusion protein consisting of the NGF binding region of trkA linked to a constant domain fragment (Fc) of an IgG antibody. TrkA-Fc produces hypoalgesia in naïve animals, decreases nociceptor responses, and decreases sprouting of unmyelinated pain-sensing neurons (Bennett et al., 1998).

Antisera raised against NGF can also reduce NGF levels when injected locally or systemically. Both anti-NGF antisera and trkA-Fc attenuate carrageenan- or CFA-induced inflammatory paw pain (Koltzenberg et al., 1999) and inflamed bladder responses in rats (Jaggar et al., 1999). Anti-NGF antiserum blocks heat and cold hyperalgesia, reverses established thermal hyperalgesia, and prevents collateral sprouting in the chronic constriction injury (CCI) model of neuropathic pain (Woolf, 1996; Ro et al., 1999). Small molecule inhibitors of the trkA-NGF interaction have also been reported. In rats, the NGF-trkA inhibitor ALE-0540 reduces hyperalgesia in a thermally-induced inflammatory pain model and in the formalin test of acute and persistent pain (Owolabi et al., 1999). ALE-0540 also reduces mechanical allodynia in the sciatic nerve injury model of neuropathic pain (Owolabi et al., 1999).

Therapeutic antibodies in general hold out the promise of a degree of target selectivity within a family of closely related receptors, receptor ligands, channels, or enzymes that is rarely attainable with small molecule drugs. NGF-mediated pain is particularly well suited to safe and effective treatment with antibodies because NGF levels increase in the periphery in response to noxious stimuli and antibodies have low blood-brain barrier permeability. Whilst

polyclonal antibodies have been shown to be effective in animal models of pain, anti-NGF monoclonal antibodies are more likely to be successfully developed as human therapeutics due to the advantages in manufacturing and characterizing a consistent, well-defined, chemical, entity. The anti-nociceptive effects of mouse anti-NGF monoclonal antibodies (Sammons et al., 2000) have been reported, but the amino acid sequences of these antibodies were not provided.

Recent evidence suggests that NGF promotes other pathologies in addition to pain. Thus, anti-NGF antibodies may also possess utility for treating other NGF-mediated diseases, including but not limited to asthma, chronic obstructive pulmonary disease, pulmonary fibrosis, other diseases of airway inflammation (Hoyle, 2003; Lommatzch et al., 2003), diabetic neuropathy (Yasuda et al., 2003), cardiac arrhythmias (WO04/032852), HIV (Garaci et al., 2003), arthritis, psoriasis and cancer (Nakagawara, 2001).

WO02/096458 relates to anti-NGF antibodies, in particular mouse monoclonal antibody 911, and use of such antibodies in treatment of various NGF-related disorders, including pain, asthma, arthritis and psoriasis. It states that the antibody 911 had no adverse effect on the immune system in an experimental mouse model of allergy. These antibodies were also described by Hongo et al., 2000.

WO04/032870 describes the pain-reducing effect of the mouse monoclonal NGF antibody mab 911 and humanized NGF antibody E3 in experimental models of post-operative pain. E3 differs from human heavy chain gamma2a constant region by 2 amino acids.

WO04/032852 describes methods for preventing sudden cardiac death and for treatment of cardiac arryhthmias using NGF antagonists.

WO 01/78698 describes the use of polyclonal antiserum to NGF to treat chronic visceral pain.

The present invention provides specific binding members for NGF, preferably human NGF. Thus, a specific binding member of the invention may bind human NGF or non-human NGF (e.g. non-human primate NGF and/or rat NGF and/or mouse NGF).

Specific binding members of the invention may be antibodies to human NGF, especially human antibodies, which may be cross-reactive with non-human NGF, including non-human primate NGF and/or mouse NGF and/or rat NGF.

A specific binding member in accordance with the present invention preferably neutralises NGF. Neutralisation means reduction or inhibition of biological activity of NGF, e.g. reduction or inhibition of NGF binding to one or more of its receptors (preferably TrkA). The reduction in biological activity may be partial or total. The degree to which an antibody neutralises NGF is referred to as its neutralising potency. Potency may be determined or measured using one or more assays known to the skilled person and/or as described or referred to herein, for example:

- "FLIPR" calcium mobilisation assay (see Example 2 herein)
- PC12 survival assay (see Example 5 herein)
- TF-1 proliferation assay (see Example 6 herein)
- Receptor binding inhibition assay (see Example 9 herein).

Assays and potencies are described in more detail elsewhere herein.

Specific binding members of the present invention may be optimised for neutralising potency. Generally potency optimisation involves mutating the sequence of a selected specific binding member (normally the variable domain sequence of an antibody) to generate a library of specific binding members, which are then assayed for potency and the more potent specific binding members are selected. Thus selected "potency-optimised" specific binding members tend to have a higher potency than the specific binding member from which the library was generated. Nevertheless, high potency specific binding members may also be obtained without optimisation, for example a high potency

specific binding member may be obtained directly from an initial screen e.g. a biochemical neutralisation assay. The present invention provides both potency-optimised and non-optimised specific binding members, as well as methods for potency optimisation from a selected specific binding member. The present invention thus allows the skilled person to generate specific binding members having high potency.

A specific binding member in accordance with the present invention preferably exhibits antihyperalgesic and/or antiallodynic activity, e.g. inhibits carrageenan-induced thermal hyperalgesia.

In some embodiments, a specific binding member of the invention comprises an antibody molecule. In other embodiments, a specific binding member of the invention comprises an antigen-binding site within a non-antibody molecule, e.g. a set of CDRs in a non-antibody protein scaffold, as discussed further below.

In various aspects and embodiments of the invention there is provided the subject-matter of the claims included below.

Preferred embodiments within the present invention are antibody molecules, whether whole antibody (e.g. IgG, such as IgG4) or antibody fragments (e.g. scFv, Fab, dAb). Preferably, an antibody molecule of the invention is a human antibody molecule. Antibody molecules comprising antibody antigen-binding sites are provided, as are antibody VH and VL domains. Within VH and VL domains are provided complementarity determining regions, ("CDRs"), and framework regions, ("FRs"), to form VH or VL domains as the case may be. An antibody antigen-binding site may consist of an antibody VH domain and/or a VL domain. All VH and VL sequences, CDR sequences, sets of CDRs and sets of HCDRs and sets of LCDRs disclosed herein represent aspects and embodiments of the invention. A "set of CDRs" comprises CDR1, CDR2 and CDR3. Thus, a set of HCDRs means HCDR1, HCDR2 and HCDR3, and a

set of LCDRs means LCDR1, LCDR2 and LCDR3. Unless otherwise stated, a "set of CDRs" includes HCDRs and LCDRs.

Examples of antibody VH and VL domains and CDRs according to the present invention are as listed in the appended sequence listing.

A number of antibody lineages are disclosed herein, defined with reference to sequences, e.g. a set of CDR sequences, optionally with one or more, e.g. one or two, or two substitutions. The preferred parent lineage is the 1021E5 lineage. The 1021E5 lineage includes the preferred antibody molecule 1133C11 and other antibody molecules of the "1133C11 lineage", including 1252A5. Also within the 1021E5 parent lineage are antibody molecules 1165D4, 1230H7 and 1152H5. The present inventors have identified the 1021E5, 1083H4 and especially the 1133C11 lineages as providing human antibody antigen-binding sites against NGF that are of particular value.

The 1133C11 lineage is defined with reference to a set of six CDR sequences of 1133C11 as follows: HCDR1 SEQ ID NO: 193, HCDR2 SEQ ID NO: 194, HCDR3 SEQ ID NO: 195, LCDR1 SEQ ID NO: 198, LCDR2 SEQ ID NO: 199, and LCDR3 SEQ ID NO: 200. The set of CDRs wherein the HCDR1 has the amino acid sequence of SEQ ID NO: 193, the HCDR2 has the amino acid sequence of SEQ ID NO: 194, the HCDR3 has the amino acid sequence of SEQ ID NO: 195, the LCDR1 has the amino acid sequence of SEQ ID NO: 198, the LCDR2 has the amino acid sequence of SEQ ID NO: 199, and the LCDR3 has the amino acid sequence of SEQ ID NO: 200, are herein referred to as the "1133C11 set of CDRs". The HCDR1, HCDR2 and HCDR3 within the 1133C11 set of CDRs are referred to as the "1133C11 set of HCDRs" and the LCDR1, LCDR2 and LCDR3 within the 1133C11 set of CDRs are referred to as the "1133C11 set of LCDRs". A set of CDRs with the 1133C11 set of CDRs, 1133C11 set of HCDRs or 1133C11 LCDRs, or one or two substitutions therein, is said to be of the 1133C11 lineage.

Other preferred lineages and sets of CDRs are defined with reference to the analogous CDRs as set out anywhere herein, including as

preferred embodiments the sets of CDRs disclosed in Table 2a (with SEQ ID NOS as set out in Table 2b). Table 2a and Table 2b show sets of CDRs (HCDRs and LCDRs) from optimised clones derived from clone 1021E5, illustrating how the CDR sequences of the optimised clones differ from those of 1021E5. A set of CDRs from Table 2a/2b includes a set of HCDRs and/or a set of LCDRs from any clone illustrated in the Table, optionally including 1021E5 itself.

Sets of CDRs of these are provided, as indicated, as are sets of CDRs with the disclosed sequences containing one or two amino acid substitutions.

The present invention also provides specific binding members and antibody molecules comprising the defined sets of CDRs, set of HCDRs or set of LCDRs, as disclosed herein, and sets of CDRs of with one or two substitutions within the disclosed set of CDRs. The relevant set of CDRs is provided within an antibody framework or other protein scaffold, e.g. fibronectin or cytochrome B (Koide et al., 1998; Nygren et al., 1997), as discussed below. Preferably antibody framework regions are employed. For example, one or more CDRs or a set of CDRs of an antibody may be grafted into a framework (e.g. human framework) to provide an antibody molecule or different antibody molecules. For example, an antibody molecule may comprise CDRs of an antibody of the 1021E5 lineage and framework regions of human germline gene segment sequences. An antibody of a lineage may be provided with a set of CDRs within a framework which may be subject to "germlining", where one or more residues within the framework are changed to match the residues at the equivalent position in the most similar human germline framework (e.g. DP10 from the VH1 family) or a framework of the λ1 family e.g. DPL5. Thus, antibody framework regions are preferably germline and/or human.

The invention provides an isolated human antibody specific for NGF, having a VH domain comprising a set of HCDRs in a human germline framework comprising DP10. Normally the specific binding member also

has a VL domain comprising a set of LCDRs, preferably in a human germline framework comprising a Vλ1, e.g. DPL5. Preferably, the CDRs are a set of CDRs disclosed herein.

By "substantially as set out" it is meant that the relevant CDR or VH or VL domain of the invention will be either identical or highly similar to the specified regions of which the sequence is set out herein. By "highly similar" it is contemplated that from 1 to 5, preferably from 1 to 4 such as 1 to 3 or 1 or 2, or 3 or 4, amino acid substitutions may be made in the CDR and/or VH or VL domain.

In one aspect, the present invention provides a specific binding member for NGF, comprising an antibody antigen-binding site which is composed of a human antibody VH domain and a human antibody VL domain and which comprises a set of CDRs, wherein the VH domain comprises HCDR1, HCDR2 and HCDR3 and the VL domain comprises LCDR1, LCDR2 and LCDR3, wherein the HCDR1 has the amino acid sequence of SEQ ID NO: 193, the HCDR2 has the amino acid sequence of SEQ ID NO: 194, the HCDR3 has the amino acid sequence of SEQ ID NO: 195, the LCDR1 has the amino acid sequence of SEQ ID NO: 198, the LCDR2 has the amino acid sequence of SEQ ID NO: 199, and the LCDR3 has the amino acid sequence of SEQ ID NO: 200; or wherein the set of CDRs contains one or two amino acid substitutions compared with this set of CDRs.

Thus, the invention provides a specific binding member for NGF, comprising an antibody antigen-binding site which is composed of a human antibody VH domain and a human antibody VL domain and which comprises a set of CDRs, wherein the set of CDRs is the 1133C11 set of CDRs or other set of CDRs disclosed herein, or a set of CDRs containing one or two substitutions compared with the 1133C11 set of CDRs or other set of CDRs disclosed herein.

In preferred embodiments, the one or two substitutions are at one or two of the following residues within the CDRs of the VH and/or VL domains, using the standard numbering of Kabat (1991).

31, 34 in HCDR1

51, 55, 56, 57, 58, 65 in HCDR2

96 in HCDR3

26, 27, 27A, 27B, 28, 29, 30 in LCDR1

56 in LCDR2

90, 94 in LCDR3.

In preferred embodiments one or two substitutions are made at one or two of the following residues within the 1133C11 set of CDRs in accordance with the identified groups of possible substitute residues:

Position of substitution	Substitute Residue selected from the group consisting of
31 in HCDR1:	A
34 in HCDR1:	V
51 in HCDR2:	V
55 in HCDR2:	N
56 in HCDR2:	A
57 in HCDR2:	V
58 in HCDR2:	S
65 in HCDR2:	D
96 in HCDR3:	N

26 in LCDR1: T
26 in LCDR1: G
27 in LCDR1: N
27 in LCDR1: R
27A in LCDR1: T
27A in LCDR1: P
27B in LCDR1: D
28 in LCDR1: T
29 in LCDR1: E
30 in LCDR1: D

56 in LCDR2: T

90 in LCDR3: A
94 in LCDR3: G.

Residue 29E within LCDR1 is a particularly preferred embodiment.

Preferred embodiments have the 1133C11 or 1252A5, 1152H5, 1165D4, 1230H7 or 1021E5 set of CDRs.

In one embodiment an isolated specific binding member comprises a set of CDRs which contains the 1133C11 set of CDRs with the amino acid sequence FNSALIS (SEQ ID NO: 532) or the amino acid sequence MISSLQP (SEQ ID NO: 533), substituted for the amino acid sequence LNPSLTA (SEQ ID NO: 531) within HCDR3.

Any set of HCDRs of the lineages disclosed herein can be provided in a VH domain that is used as a specific binding member alone or in combination with a VL domain. A VH domain may be provided with a set of HCDRs of a 1133C11, 1021E5 or other lineage antibody, e.g. a set of HCDRs as illustrated in Table 2a/2b, and if such a VH domain is paired with a VL domain, then the VL domain may be provided with a set of LCDRs of a 1133C11, 1021E5 or other lineage antibody, e.g. a set of

LCDRs as illustrated in Table 2a/2b. A pairing of a set of HCDRs and a set of LCDRs may be as shown in Table 2a/2b, providing an antibody antigen-binding site comprising a set of CDRs as shown in Table 2a/2b.

The VH and VL domain frameworks comprise framework regions, one or more of which may be a germlined framework region, normally human germline. The VH domain framework is preferably human heavy chain germ-line framework and the VL domain framework is preferably human light chain germ-line framework. Framework regions of the heavy chain domain may be selected from the VH-1 family, and a preferred VH-1 framework is a DP-10 framework. Framework regions of the light chain may be selected from the λ1 family, and a preferred framework is DPL5.

One or more CDRs may be taken from the 1252A5 VH or VL domain and incorporated into a suitable framework. This is discussed further herein. 1252A5 HCDRs 1, 2 and 3 are shown in SEQ ID NO: 393, 394, 395 respectively. 1252A5 LCDRs 1, 2 and 3 are shown in SEQ ID NO: 398, 399, 400, respectively.

All this applies the same for other CDRs and sets of CDRs as disclosed herein, especially for 1152H5, 1165D4 and 1230H7.

Embodiments of the present invention employ the antibody VH and/or VL domain of an antibody molecule of the 1021E5 lineage, e.g. the antibody molecule 1021E5. A specific binding member comprising an antibody antigen-binding site comprising such a VH and/or VL domain is also provided by the present invention.

Preferred embodiments are as follows:

A VH domain, VL domain, set of HCDRs, set of LCDRs, or set of CDRs of: 1126F1 (VH SEQ ID NO: 102; VL SEQ ID NO: 107), 1126G5 (VH SEQ ID NO: 112; VL SEQ ID NO: 117), 1126H5 (VH SEQ ID NO: 122; VL SEQ ID NO: 127), 1127D9 (VH SEQ ID NO: 132; VL SEQ ID NO: 137), 1127F9 (VH SEQ ID

NO: 142; VL SEQ ID NO: 147), 1131D7 (VH SEQ ID NO: 152; VL SEQ ID NO: 157), 1131H2 (VH SEQ ID NO: 162; VL SEQ ID NO: 167), 1132A9 (VH SEQ ID NO: 172; VL SEQ ID NO: 177), 1132H9 (VH SEQ ID NO: 182; VL SEQ ID NO: 187), 1133C11 (VH SEQ ID NO: 192; VL SEQ ID NO: 197), 1134D9 (VH SEQ ID NO: 202; VL SEQ ID NO: 207), 1145D1 (VH SEQ ID NO: 212; VL SEQ ID NO: 217), 1146D7 (VH SEQ ID NO: 222; VL SEQ ID NO: 227), 1147D2 (VH SEQ ID NO: 232; VL SEQ ID NO: 237), 1147G9 (VH SEQ ID NO: 242; VL SEQ ID NO: 247), 1150F1 (VH SEQ ID NO: 252; VL SEQ ID NO: 257), 1152H5 (VH SEQ ID NO: 262; VL SEQ ID NO: 267), 1155H1 (VH SEQ ID NO: 272; VL SEQ ID NO: 277), 1158A1 (VH SEQ ID NO: 282; VL SEQ ID NO: 287), 1160E3 (VH SEQ ID NO: 292; VL SEQ ID NO: 297), 1165D4 (VH SEQ ID NO: 302; VL SEQ ID NO: 307), 1175H8 (VH SEQ ID NO: 312; VL SEQ ID NO: 317), 1211G10 (VH SEQ ID NO: 322; VL SEQ ID NO: 327), 1214A1 (VH SEQ ID NO: 332; VL SEQ ID NO: 337), 1214D10 (VH SEQ ID NO: 342; VL SEQ ID NO: 347), 1218H5 (VH SEQ ID NO: 352; VL SEQ ID NO: 357), and 1230H7 (VH SEQ ID NO: 362; VL SEQ ID NO: 367).

Still further preferred are a VH domain, VL domain, set of HCDRs, set of LCDRs, or set of CDRs of 1083H4 (VH SEQ ID NO: 22; VL SEQ ID NO: 27), 1227H8 (VH SEQ ID NO: 372; VL SEQ ID NO: 377) and 1230D8 (VH SEQ ID NO: 382; VL SEQ ID NO: 387).

In a highly preferred embodiment, a VH domain is provided with the amino acid sequence of SEQ ID NO: 192, this being termed "1133C11 VH domain". In a further highly preferred embodiment, a VL domain is provided with the amino acid sequence of SEQ ID NO: 197, this being termed "1133C11 VL domain". A highly preferred antibody antigen-binding site provided in accordance with the present invention is composed of the 1133C11 VH domain, SEQ ID NO: 192, and the 1133C11 VL domain, SEQ ID NO: 197. This antibody antigen-binding site may be provided within any desired antibody molecule format, e.g. scFv, Fab, IgG, IgG4 etc., as is discussed further elsewhere herein.

In a further highly preferred embodiment, a VH domain is provided with the amino acid sequence of SEQ ID NO: 392, this being termed "1252A5

VH domain". In a further highly preferred embodiment, a VL domain is provided with the amino acid sequence of SEQ ID NO: 397, this being termed "1252A5 VL domain". A highly preferred antibody antigen-binding site provided in accordance with the present invention is composed of the 1252A5 VH domain, SEQ ID NO: 392, and the 1252A5 VL domain, SEQ ID NO: 397. This antibody antigen-binding site may be provided within any desired antibody molecule format, e.g. scFv, Fab, IgG, IgG4 etc., as is discussed further elsewhere herein.

In a further highly preferred embodiment, the present invention provides an IgG4 antibody molecule comprising the 1252A5 VH domain, SEQ ID NO: 392, and the 1252A5 VL domain, SEQ ID NO: 397. This is termed herein "1252A5 IgG4".

Other IgG or other antibody molecules comprising the 1252A5 VH domain, SEQ ID NO: 392, and/or the 1252A5 VL domain, SEQ ID NO: 397, are provided by the present invention, as are other antibody molecules comprising the 1252A5 set of HCDRs (SEQ ID NOS: 393, 394 and 395) within an antibody VH domain, and/or the 1252A5 set of LCDRs (SEQ ID NOS: 398, 399 and 400) within an antibody VL domain.

As noted, the present invention provides a specific binding member which binds human NGF and which comprises the 1252A5 VH domain (SEQ ID NO: 392) and/or the 1252A5 VL domain (SEQ ID NO: 397). Properties of such a specific binding member are disclosed herein.

Generally, a VH domain is paired with a VL domain to provide an antibody antigen-binding site, although as discussed further below a VH domain alone may be used to bind antigen. In one preferred embodiment, the 1252A5 VH domain (SEQ ID NO: 392) is paired with the 1252A5 VL domain (SEQ ID NO: 397), so that an antibody antigen-binding site is formed comprising both the 1252A5 VH and VL domains. Analogous embodiments are provided for the other VH and VL domains disclosed herein. In other embodiments, the 1252A5 VH is paired with a VL domain other than the 1252A5 VL. Light-chain promiscuity is well

established in the art. Again, analogous embodiments are provided by the invention for the other VH and VL domains disclosed herein.

Variants of the VH and VL domains and CDRs of the present invention, including those for which amino acid sequences are set out herein, and which can be employed in specific binding members for NGF can be obtained by means of methods of sequence alteration or mutation and screening. Such methods are also provided by the present invention.

In accordance with further aspects of the present invention there is provided a specific binding member which competes for binding to antigen with any specific binding member which both binds the antigen and comprises a specific binding member, VH and/or VL domain disclosed herein, or HCDR3 disclosed herein, or variant of any of these. Competition between binding members may be assayed easily *in vitro*, for example using ELISA and/or by tagging a specific reporter molecule to one binding member which can be detected in the presence of one or more other untagged binding members, to enable identification of specific binding members which bind the same epitope or an overlapping epitope.

Thus, a further aspect of the present invention provides a specific binding member comprising a human antibody antigen-binding site that competes with an antibody molecule, for example especially 1252A5 or other preferred scFv and/or IgG4, for binding to NGF. In further aspects the present invention provides a specific binding member comprising a human antibody antigen-binding site which competes with an antibody antigen-binding site for binding to NGF, wherein the antibody antigen-binding site is composed of a VH domain and a VL domain, and wherein the VH and VL domains comprise a set of CDRs of the 1133C11, 1021E5, 1252A5 or other lineage, disclosed herein.

Various methods are available in the art for obtaining antibodies against NGF and which may compete with a 1252A5 or other antibody molecule, an antibody molecule with a 1252A5 or other set of CDRs, or

an antibody molecule with a set of CDRs of 1252A5 or other lineage, for binding to NGF.

In a further aspect, the present invention provides a method of obtaining one or more specific binding members able to bind the antigen, the method including bringing into contact a library of specific binding members according to the invention and said antigen, and selecting one or more specific binding members of the library able to bind said antigen.

The library may be displayed on particles or molecular complexes, e.g. replicable genetic packages such as yeast, bacterial or bacteriophage (e.g. T7) particles, or covalent, ribosomal or other *in vitro* display systems, each particle or molecular complex containing nucleic acid encoding the antibody VH variable domain displayed on it, and optionally also a displayed VL domain if present.

Following selection of specific binding members able to bind the antigen and displayed on bacteriophage or other library particles or molecular complexes, nucleic acid may be taken from a bacteriophage or other particle or molecular complex displaying a said selected specific binding member. Such nucleic acid may be used in subsequent production of a specific binding member or an antibody VH or VL variable domain by expression from nucleic acid with the sequence of nucleic acid taken from a bacteriophage or other particle or molecular complex displaying a said selected specific binding member.

An antibody VH variable domain with the amino acid sequence of an antibody VH variable domain of a said selected specific binding member may be provided in isolated form, as may a specific binding member comprising such a VH domain.

Ability to bind NGF may be further tested, also ability to compete with e.g. 1252A5 (e.g. in scFv format and/or IgG format, e.g. IgG4)

for binding to NGF. Ability to neutralise NGF may be tested, as discussed further below.

A specific binding member according to the present invention may bind NGF with the affinity of a 1252A5 or other antibody molecule, e.g. scFv, or preferably 1252A5 or other IgG4, or with an affinity that is better.

A specific binding member according to the present invention may neutralise NGF with the potency of a 1252A5 or other antibody molecule, e.g. scFv, or preferably 1252A5 or other IgG4, or with a potency that is better.

Binding affinity and neutralisation potency of different specific binding members can be compared under appropriate conditions.

The antibodies of the present invention have a number of advantages over existing commercially available anti-NGF antibodies. For example, the present invention provides human or germlined antibodies, which are expected to display a lower degree of immunogenicity when chronically or repeatedly administered to humans for therapeutic or diagnostic use. Further, the present invention provides antibodies that are more potent neutralisers of NGF and therefore a desired therapeutic or diagnostic effect may be achieved using less antibody material. In addition, in one embodiment of the invention, the potency for inhibition of the NGF/TrKA receptor interaction is greater than that observed for inhibition of the NGF/p75 receptor interaction. This may confer advantages over other apparently non-selective NGF antagonist treatments in this regard, either in the magnitude or nature of the therapeutic effect achieved, or in reducing undesirable side effects.

The invention also provides heterogeneous preparations comprising anti-NGF antibody molecules. For example, such preparations may be mixtures of antibodies with full-length heavy chains and heavy chains

lacking the C-terminal lysine, with various degrees of glycosylation and/or with derivatized amino acids, such as cyclization of an N-terminal glutamic acid to form a pyroglutamic acid residue.

In further aspects, the invention provides an isolated nucleic acid which comprises a sequence encoding a specific binding member, VH domain and/or VL domains according to the present invention, and methods of preparing a specific binding member, a VH domain and/or a VL domain of the invention, which comprise expressing said nucleic acid under conditions to bring about production of said specific binding member, VH domain and/or VL domain, and recovering it.

A further aspect of the present invention provides nucleic acid, generally isolated, encoding an antibody VH variable domain and/or VL variable domain disclosed herein.

Another aspect of the present invention provides nucleic acid, generally isolated, encoding a VH CDR or VL CDR sequence disclosed herein, especially a VH CDR selected from: 1133C11 (VH CDR1 SEQ ID NO: 193, VH CDR2 SEQ ID NO: 194, and VH CDR3 SEQ ID NO: 195), 1152H5(VH CDR1 SEQ ID NO: 263, VH CDR2 SEQ ID NO: 264, and VH CDR3 SEQ ID NO: 265), and 1252A5 (VH CDR1 SEQ ID NO: 393, VH CDR2 SEQ ID NO: 394, and VH CDR3 SEQ ID NO: 395), or a VL CDR selected from: 1133C11(VL CDR1 SEQ ID NO: 198, VL CDR2 SEQ ID NO: 199, and VL CDR3 SEQ ID NO: 200), 1152H5 (VL CDR1 SEQ ID NO: 268, VL CDR2 SEQ ID NO: 269, and VL CDR3 SEQ ID NO: 270), and 1252A5 (VL CDR1 SEQ ID NO: 398, VL CDR2 SEQ ID NO: 399, and VL CDR3 SEQ ID NO: 400), most preferably 1252A5 VH CDR3 (SEQ ID NO: 395). Nucleic acid encoding the 1252A5 set of CDRs, nucleic acid encoding the 1252A5 set of HCDRs and nucleic acid encoding the 1252A5 set of LCDRs are also provided by the present invention, as are nucleic acids encoding individual CDRs, HCDRs, LCDRs and sets of CDRs, HCDRs, LCDRs of the 1252A5, 1133C11 or 1021E5 lineage.

A further aspect provides a host cell transformed with nucleic acid of the invention.

A yet further aspect provides a method of production of an antibody VH variable domain, the method including causing expression from encoding nucleic acid. Such a method may comprise culturing host cells under conditions for production of said antibody VH variable domain.

Analogous methods for production of VL variable domains and specific binding members comprising a VH and/or VL domain are provided as further aspects of the present invention.

A method of production may comprise a step of isolation and/or purification of the product. A method of production may comprise formulating the product into a composition including at least one additional component, such as a pharmaceutically acceptable excipient.

Further aspects of the present invention provide for compositions containing specific binding members of the invention, and their use in methods of inhibiting or neutralising NGF, including methods of treatment of the human or animal body by therapy.

Specific binding members according to the invention may be used in a method of treatment or diagnosis of the human or animal body, such as a method of treatment (which may include prophylactic treatment) of a disease or disorder in a human patient which comprises administering to said patient an effective amount of a specific binding member of the invention. Conditions treatable in accordance with the present invention include any in which NGF plays a role, especially pain, asthma, chronic obstructive pulmonary disease, pulmonary fibrosis, other diseases of airway inflammation, diabetic neuropathy, HIV, cardiac arrhythmias, arthritis, psoriasis and cancer.

These and other aspects of the invention are described in further detail below.

TERMINOLOGY

It is convenient to point out here that "and/or" where used herein is to be taken as specific disclosure of each of the two specified features or components with or without the other. For example "A and/or B" is to be taken as specific disclosure of each of (i) A, (ii) B and (iii) A and B, just as if each is set out individually herein.

NGF

NGF (also known as beta-NGF) is nerve growth factor. In the context of the present invention, NGF is normally human NGF, although it may be non-human NGF (e.g. non-human primate NGF and/or rat NGF and/or mouse NGF). NGF is also referred to in places as "the antigen".

NGF used in an assay described herein is normally human, rat or mouse NGF, but NGF from another non-human animal could be used, e.g. non-human primate NGF.

Pain

This describes, as is well known in the art, sensation of pain, and may encompass one or more, or all, of the following:

- hyperalgesia (exaggerated pain response to a normally painful stimulus);
- allodynia (sensation of pain caused by a stimulus that is not normally painful);
- spontaneous sensation of pain caused by any mechanism in the absence of any apparent external influence;
- pain evoked by physical stimuli, such as heat, warmth, cold, pressure, vibration, static or dynamic touch, or body posture and movement;
- somatic and visceral pain caused by any mechanism, for example, trauma, infection, inflammation, metabolic disease, stroke or neurological disease.

Pain may for example be acute pain, short-term pain, persistent nociceptive pain, or persistent or chronic neuropathic pain.

Specific binding member

This describes a member of a pair of molecules that have binding specificity for one another. The members of a specific binding pair may be naturally derived or wholly or partially synthetically produced. One member of the pair of molecules has an area on its surface, or a cavity, which specifically binds to and is therefore complementary to a particular spatial and polar organisation of the other member of the pair of molecules. Thus the members of the pair have the property of binding specifically to each other. Examples of types of specific binding pairs are antigen-antibody, biotin-avidin, hormone-hormone receptor, receptor-ligand, enzyme-substrate. The present invention is concerned with antigen-antibody type reactions.

A specific binding member normally comprises a molecule having an antigen-binding site. For example, a specific binding member may be an antibody molecule or a non-antibody protein that comprises an antigen-binding site. An antigen binding site may be provided by means of arrangement of CDRs on non-antibody protein scaffolds such as fibronectin or cytochrome B etc. (Haan & Maggos, 2004; Koide et al., 1998; Nygren et al., 1997), or by randomising or mutating amino acid residues of a loop within a protein scaffold to confer binding specificity for a desired target. Scaffolds for engineering novel binding sites in proteins have been reviewed in detail by Nygren et al. (1997). Protein scaffolds for antibody mimics are disclosed in WO/0034784 in which the inventors describe proteins (antibody mimics) that include a fibronectin type III domain having at least one randomised loop. A suitable scaffold into which to graft one or more CDRs, e.g. a set of HCDRs, may be provided by any domain member of the immunoglobulin gene superfamily. The scaffold may be a human or non-human protein.

An advantage of a non-antibody protein scaffold is that it may provide an antigen-binding site in a scaffold molecule that is smaller and/or easier to manufacture than at least some antibody molecules. Small size of a specific binding member may confer useful physiological properties such as an ability to enter cells, penetrate deep into tissues or reach targets within other structures, or to bind within protein cavities of the target antigen.

Use of antigen binding sites in non-antibody protein scaffolds is reviewed in Wess, 2004. Typical are proteins having a stable backbone and one or more variable loops, in which the amino acid sequence of the loop or loops is specifically or randomly mutated to create an antigen-binding site having specificity for binding the target antigen. Such proteins include the IgG-binding domains of protein A from *S. aureus*, transferrin, tetranectin, fibronectin (e.g. 10th fibronectin type III domain) and lipocalins. Other approaches include synthetic "Microbodies" (Selecore GmbH), which are based on cyclotides - small proteins having intra-molecular disulphide bonds.

In addition to antibody sequences and/or an antigen-binding site, a specific binding member according to the present invention may comprise other amino acids, e.g. forming a peptide or polypeptide, such as a folded domain, or to impart to the molecule another functional characteristic in addition to ability to bind antigen. Specific binding members of the invention may carry a detectable label, or may be conjugated to a toxin or a targeting moiety or enzyme (e.g. via a peptidyl bond or linker). For example, a specific binding member may comprise a catalytic site (e.g. in an enzyme domain) as well as an antigen binding site, wherein the antigen binding site binds to the antigen and thus targets the catalytic site to the antigen. The catalytic site may inhibit biological function of the antigen, e.g. by cleavage.

Although, as noted, CDRs can be carried by scaffolds such as fibronectin or cytochrome B (Haan & Maggos, 2004; Koide et al., 1998;

Nygren et al., 1997), the structure for carrying a CDR or a set of CDRs of the invention will generally be of an antibody heavy or light chain sequence or substantial portion thereof in which the CDR or set of CDRs is located at a location corresponding to the CDR or set of CDRs of naturally occurring VH and VL antibody variable domains encoded by rearranged immunoglobulin genes. The structures and locations of immunoglobulin variable domains may be determined by reference to (Kabat, et al., 1987, and updates thereof, now available on the Internet (http://immuno.bme.nwu.edu or find "Kabat" using any search engine).

Antibody molecule

This describes an immunoglobulin whether natural or partly or wholly synthetically produced. The term also covers any polypeptide or protein comprising an antibody antigen-binding site. Antibody fragments that comprise an antibody antigen-binding site are molecules such as Fab, scFv, Fv, dAb, Fd; and diabodies.

It is possible to take monoclonal and other antibodies and use techniques of recombinant DNA technology to produce other antibodies or chimeric molecules that retain the specificity of the original antibody. Such techniques may involve introducing DNA encoding the immunoglobulin variable region, or the CDRs, of an antibody to the constant regions, or constant regions plus framework regions, of a different immunoglobulin. See, for instance, EP-A-184187, GB 2188638A or EP-A-239400, and a large body of subsequent literature. A hybridoma or other cell producing an antibody may be subject to genetic mutation or other changes, which may or may not alter the binding specificity of antibodies produced.

As antibodies can be modified in a number of ways, the term "antibody molecule" should be construed as covering any specific binding member or substance having an antibody antigen-binding site with the required specificity. Thus, this term covers antibody fragments and derivatives, including any polypeptide comprising an antibody antigen-

binding site, whether natural or wholly or partially synthetic. Chimeric molecules comprising an antibody antigen-binding site, or equivalent, fused to another polypeptide are therefore included. Cloning and expression of chimeric antibodies are described in EP-A-0120694 and EP-A-0125023, and a large body of subsequent literature.

Further techniques available in the art of antibody engineering have made it possible to isolate human and humanised antibodies. For example, human hybridomas can be made as described by Kontermann & Dubel (2001). Phage display, another established technique for generating specific binding members has been described in detail in many publications such as Kontermann & Dubel (2001) and WO92/01047 (discussed further below). Transgenic mice in which the mouse antibody genes are inactivated and functionally replaced with human antibody genes while leaving intact other components of the mouse immune system, can be used for isolating human antibodies (Mendez et al., 1997).

Synthetic antibody molecules may be created by expression from genes generated by means of oligonucleotides synthesized and assembled within suitable expression vectors, for example as described by Knappik et al. (2000) or Krebs et al. (2001).

It has been shown that fragments of a whole antibody can perform the function of binding antigens. Examples of binding fragments are (i) the Fab fragment consisting of VL, VH, CL and CH1 domains; (ii) the Fd fragment consisting of the VH and CH1 domains; (iii) the Fv fragment consisting of the VL and VH domains of a single antibody; (iv) the dAb fragment (Ward, 1989; McCafferty et al., 1990; Holt et al., 2003), which consists of a VH or a VL domain; (v) isolated CDR regions; (vi) F(ab')2 fragments, a bivalent fragment comprising two linked Fab fragments (vii) single chain Fv molecules (scFv), wherein a VH domain and a VL domain are linked by a peptide linker which allows the two domains to associate to form an antigen binding site (Bird et al., 1988; Huston et al., 1988); (viii) bispecific single chain Fv dimers

(PCT/US92/09965) and (ix) "diabodies", multivalent or multispecific fragments constructed by gene fusion (WO94/13804; Holliger et al., 1993). Fv, scFv or diabody molecules may be stabilised by the incorporation of disulphide bridges linking the VH and VL domains (Reiter et al., 1996). Minibodies comprising a scFv joined to a CH3 domain may also be made (Hu et al., 1996).

A dAb (domain antibody) is a small monomeric antigen-binding fragment of an antibody, namely the variable region of an antibody heavy or light chain (Holt *et al.*, 2003). VH dAbs occur naturally in camelids (e.g. camel, llama) and may be produced by immunising a camelid with a target antigen, isolating antigen-specific B cells and directly cloning dAb genes from individual B cells. dAbs are also producible in cell culture. Their small size, good solubility and temperature stability makes them particularly physiologically useful and suitable for selection and affinity maturation. A specific binding member of the present invention may be a dAb comprising a VH or VL domain substantially as set out herein, or a VH or VL domain comprising a set of CDRs substantially as set out herein.

Where bispecific antibodies are to be used, these may be conventional bispecific antibodies, which can be manufactured in a variety of ways (Holliger & Winter, 1993), e.g. prepared chemically or from hybrid hybridomas, or may be any of the bispecific antibody fragments mentioned above. Examples of bispecific antibodies include those of the BiTE™ technology in which the binding domains of two antibodies with different specificity can be used and directly linked via short flexible peptides. This combines two antibodies on a short single polypeptide chain. Diabodies and scFv can be constructed without an Fc region, using only variable domains, potentially reducing the effects of anti-idiotypic reaction.

Bispecific diabodies, as opposed to bispecific whole antibodies, may also be particularly useful because they can be readily constructed and expressed in *E.coli*. Diabodies (and many other polypeptides such

as antibody fragments) of appropriate binding specificities can be readily selected using phage display (WO94/13804) from libraries. If one arm of the diabody is to be kept constant, for instance, with a specificity directed against NGF, then a library can be made where the other arm is varied and an antibody of appropriate specificity selected. Bispecific whole antibodies may be made by knobs-into-holes engineering (Ridgeway et al., 1996).

Antigen-binding site

This describes the part of a molecule that binds to and is complementary to all or part of the target antigen. In an antibody molecule it is referred to as the antibody antigen-binding site, and comprises the part of the antibody that specifically binds to and is complementary to all or part of the target antigen. Where an antigen is large, an antibody may only bind to a particular part of the antigen, which part is termed an epitope. An antibody antigen-binding site may be provided by one or more antibody variable domains. Preferably, an antibody antigen-binding site comprises an antibody light chain variable region (VL) and an antibody heavy chain variable region (VH).

Specific

This may be used to refer to the situation in which one member of a specific binding pair will not show any significant binding to molecules other than its specific binding partner(s). The term is also applicable where e.g. an antigen-binding site is specific for a particular epitope that is carried by a number of antigens, in which case the specific binding member carrying the antigen-binding site will be able to bind to the various antigens carrying the epitope.

Isolated

This refers to the state in which specific binding members of the invention, or nucleic acid encoding such binding members, will generally be in accordance with the present invention. Isolated members and isolated nucleic acid will be free or substantially free

of material with which they are naturally associated such as other polypeptides or nucleic acids with which they are found in their natural environment, or the environment in which they are prepared (e.g. cell culture) when such preparation is by recombinant DNA technology practised *in vitro* or *in vivo*. Members and nucleic acid may be formulated with diluents or adjuvants and still for practical purposes be isolated - for example the members will normally be mixed with gelatin or other carriers if used to coat microtitre plates for use in immunoassays, or will be mixed with pharmaceutically acceptable carriers or diluents when used in diagnosis or therapy. Specific binding members may be glycosylated, either naturally or by systems of heterologous eukaryotic cells (e.g. CHO or NS0 (ECACC 85110503) cells, or they may be (for example if produced by expression in a prokaryotic cell) unglycosylated.

DETAILED DESCRIPTION

As noted above, a specific binding member in accordance with the present invention preferably neutralises NGF. The degree to which an antibody neutralises NGF is referred to as its neutralising potency.

Potency is normally expressed as an IC50 value, in nM unless otherwise stated. IC50 is the median inhibitory concentration of an antibody molecule. In functional assays, IC50 is the concentration that reduces a biological response by 50 % of its maximum. In ligand-binding studies, IC50 is the concentration that reduces receptor binding by 50 % of maximal specific binding level.

IC50 may be calculated by plotting % biological response (represented e.g. by calcium ion mobilisation in a FLIPR assay, by survival in a PC12 assay, or by proliferation in a TF-1 proliferation assay) or % specific receptor binding as a function of the log of the specific binding member concentration, and using a software program such as Prism (GraphPad) to fit a sigmoidal function to the data to generate IC50 values, for example as described in Example 2, 5, 6 or 9 herein.

A specific binding member in accordance with the present invention preferably inhibits human NGF-evoked intracellular calcium mobilisation in cells expressing TrkA receptor, e.g. cells recombinantly transfected with a TrkA gene, for instance HEK cells. In a "FLIPR" calcium mobilisation assay as described in Example 2 herein, a specific binding member according to the invention preferably has a potency (IC50) for neutralising human NGF of or less than 600, 100, 90, 80, 70, 60, 50, 40, 30, 20 or 10 nM. Normally, a specific binding member of the invention has a potency of 5 nM or less, preferably 2.5 nM or less, more preferably 1 nM or less. In particularly preferred embodiments, the potency is 0.5 nM or less, e.g. 0.4 nM or less; 0.3 nM or less; 0.2 nM or less; or 0.15 nM or less. In some embodiments, the potency may be about 0.1 nM.

Potency may be between 0.1-100 nM, 0.1-50 nM, 0.1-10 nM, or 0.1-1.0 nM. For example, potency may be 0.1-5.0 nM, 0.2-5.0 nM, 0.3-5.0 nM, or 0.3-0.4 nM.

In some embodiments of the invention, the neutralising potency of a non potency-optimised specific binding member in a HEK cell assay as described herein is about 1.8 to 560 nM for human NGF and/or about 2.9 to 620 nM for rat NGF. In some embodiments, the neutralizing potency of potency-optimised binding members in HEK cell assays as described herein are about 0.12 to 120nM for human NGF, about 0.11 to 37 nM for rat NGF and about 0.11 to 71 nM for mouse NGF. However, these are examples only and higher potencies may be achieved. Although potency optimisation may be used to generate higher potency specific binding members from a given specific binding member, it is also noted that high potency specific binding members may be obtained even without potency optimisation.

A specific binding member in accordance with the present invention preferably inhibits NGF-maintained serum-deprived PC12 cell survival. The neutralising potency of a specific binding member of the present

invention in a PC12 survival assay for human NGF as described herein in Example 5 is generally 1500 nM or less, and is preferably 50 nM or less, or 10 nM or less. As explained above and as demonstrated herein, potency-optimisation may be used to achieve higher anti-NGF potencies. Preferably, a specific binding member has a potency of or less than 5 nM, 4 nM, 3 nM, 2 nM, 1.5 nM, 1 nM or 0.5 nM. In some embodiments, potency is about 0.1 nM or more, 0.2 nM or more. Thus, potency may be between 0.1 or 0.2 nM and 0.5, 1.5, 5 or 50 nM.

In some embodiments of the invention, the neutralizing potency of a potency optimised specific binding member in a PC12 survival assay as described herein is about 0.2 to 670nM for human NGF and is about 0.2 to 54 nM for rat NGF.

A specific binding member in accordance with the present invention preferably inhibits NGF-stimulated TF-1 cell proliferation. The neutralising potency of a specific binding member (normally a potency-optimised specific binding member) of the present invention in a TF-1 proliferation assay for human NGF as described herein in Example 6 is generally 5 nM or less, preferably 1 nM or less. Preferably, a specific binding member of the invention has a potency of or less than 0.7, 0.6, 0.5, 0.45, 0.4, 0.3, 0.2 or 0.1 nM for human NGF. For example, potency may be between 0.05 - 0.1 nm, 0.05 - 0.2 nM, 0.05 - 0.3 nM, 0.05 - 0.4 nM, or 0.05 - 0.5 nM.

In some embodiments of the invention, the neutralizing potency of a potency optimised specific binding member in a TF-1 proliferation assay as described herein is about 0.08 to 0.7nM for human NGF, about 0.07 to 1.9 nM for rat NGF and about 0.07 to 1.4nM for mouse NGF.

A specific binding member in accordance with the present invention preferably inhibits NGF binding to a TrkA and/or p75 receptor, preferably a human TrkA and/or p75 receptor. The invention also extends more generally to a specific binding member that preferentially blocks NGF binding to TrkA receptor over NGF binding to

p75 receptor. The neutralising potency of a specific binding member (normally a potency-optimised specific binding member) of the present invention in a TrkA receptor binding assay as described herein in Example 9 is generally 2.5 nM or less, preferably 1 nM or less for neutralising human NGF. Preferably, a specific binding member of the invention has a potency of or less than 0.5, 0.4, 0.3, 0.2, 0.1 or 0.075 nM for neutralising human NGF binding to TrkA. For example, potency may be between 0.05 - 0.1 nm, 0.05 - 0.2 nM, 0.05 - 0.3 nM, 0.05 - 0.4 nM, or 0.05 - 0.5 nM.

The neutralising potency of a specific binding member (normally a potency-optimised specific binding member) of the present invention in a p75 receptor binding assay as described herein in Example 9 is generally 1.5 nM or less, preferably 1 nM or less for neutralising human NGF. Preferably, a specific binding member of the invention has a potency of or less than 0.9, 0.8, 0.7, 0.6, 0.5, 0.4, 0.3, 0.2 or 0.1 nM for neutralising human NGF binding to p75. For example, potency may be between 0.1 - 0.2 nM, 0.1 - 0.3 nM, 0.1 - 0.4 nM, 0.1 - 0.5 nM, or 0.1-0.6 nM.

Some preferred specific binding members according to the present invention inhibit NGF (e.g. human and/or rat NGF) binding to TrkA receptor preferentially over NGF binding to p75 receptor. Accordingly, in some embodiments a specific binding member of the invention has a lower binding inhibition constant, Ki, for inhibition of NGF (e.g. human and/or rat NGF) binding to TrkA than for NGF binding to p75. Ki may be calculated using the formula set out in Example 9. Alternatively, binding inhibition constants can be expressed as pKi, which can be calculated as $-\log_{10}$Ki. Thus, a specific binding member of the invention preferably has a higher pKi value for inhibition of NGF binding to TrkA than to p75.

Preferably, a specific binding member according to the invention binds human NGF and/or rat NGF with an affinity of or less than 1, 0.8, 0.7, 0.6, 0.5, 0.4, 0.3 or 0.2 nM. For example, a specific binding member

may bind human NGF with an affinity of about 0.25-0.44nM and rat NGF with an affinity of about 0.25-0.70 nM.

As noted above, variants of antibody molecules disclosed herein may be produced and used in the present invention. Following the lead of computational chemistry in applying multivariate data analysis techniques to the structure/property-activity relationships (Wold, *et al.* 1984) quantitative activity-property relationships of antibodies can be derived using well-known mathematical techniques such as statistical regression, pattern recognition and classification (Norman *et al.* 1998; Kandel & Backer, 1995; Krzanowski, 2000; Witten & Frank, 1999; Denison (Ed), 2002; Ghose & Viswanadhan). The properties of antibodies can be derived from empirical and theoretical models (for example, analysis of likely contact residues or calculated physicochemical property) of antibody sequence, functional and three-dimensional structures and these properties can be considered singly and in combination.

An antibody antigen-binding site composed of a VH domain and a VL domain is formed by six loops of polypeptide: three from the light chain variable domain (VL) and three from the heavy chain variable domain (VH). Analysis of antibodies of known atomic structure has elucidated relationships between the sequence and three-dimensional structure of antibody combining sites (Chothia et al. 1992; Al-Lazikani, *et al.* 1997). These relationships imply that, except for the third region (loop) in VH domains, binding site loops have one of a small number of main-chain conformations: canonical structures. The canonical structure formed in a particular loop has been shown to be determined by its size and the presence of certain residues at key sites in both the loop and in framework regions (Chothia *et al.* and Al-Lazikani *et al.*, supra).

This study of sequence-structure relationship can be used for prediction of those residues in an antibody of known sequence, but of an unknown three-dimensional structure, which are important in

maintaining the three-dimensional structure of its CDR loops and hence maintain binding specificity. These predictions can be backed up by comparison of the predictions to the output from lead optimization experiments. In a structural approach, a model can be created of the antibody molecule (Chothia, *et al.* 1986) using any freely available or commercial package such as WAM (Whitelegg & Rees, 2000). A protein visualisation and analysis software package such as Insight II (Accelerys, Inc.) or Deep View (Guex & Peitsch, 1997) may then be used to evaluate possible substitutions at each position in the CDR. This information may then be used to make substitutions likely to have a minimal or beneficial effect on activity.

The techniques required to make substitutions within amino acid sequences of CDRs, antibody VH or VL domains and specific binding members generally are available in the art. Variant sequences may be made, with substitutions that may or may not be predicted to have a minimal or beneficial effect on activity, and tested for ability to bind and/or neutralise NGF and/or for any other desired property.

Variable domain amino acid sequence variants of any of the VH and VL domains whose sequences are specifically disclosed herein may be employed in accordance with the present invention, as discussed. Particular variants may include one or more amino acid sequence alterations (addition, deletion, substitution and/or insertion of an amino acid residue), may be less than about 20 alterations, less than about 15 alterations, less than about 10 alterations or less than about 5 alterations, maybe 5, 4, 3, 2 or 1. Alterations may be made in one or more framework regions and/or one or more CDRs.

Preferably alterations do not result in loss of function, so a specific binding member comprising a thus-altered amino acid sequence preferably retains an ability to bind and/or neutralise NGF. More preferably, it retains the same quantitative binding and/or neutralising ability as a specific binding member in which the alteration is not made, e.g. as measured in an assay described herein.

Most preferably, the specific binding member comprising a thus-altered amino acid sequence has an improved ability to bind or neutralise NGF.

Alteration may comprise replacing one or more amino acid residue with a non-naturally occurring or non-standard amino acid, modifying one or more amino acid residue into a non-naturally occurring or non-standard form, or inserting one or more non-naturally occurring or non-standard amino acid into the sequence. Preferred numbers and locations of alterations in sequences of the invention are described elsewhere herein. Naturally occurring amino acids include the 20 "standard" L-amino acids identified as G, A, V, L, I, M, P, F, W, S, T, N, Q, Y, C, K, R, H, D, E by their standard single-letter codes. Non-standard amino acids include any other residue that may be incorporated into a polypeptide backbone or result from modification of an existing amino acid residue. Non-standard amino acids may be naturally occurring or non-naturally occurring. Several naturally occurring non-standard amino acids are known in the art, such as 4-hydroxyproline, 5-hydroxylysine, 3-methylhistidine, N-acetylserine, etc. (Voet & Voet, 1995). Those amino acid residues that are derivatised at their N-alpha position will only be located at the N-terminus of an amino-acid sequence. Normally in the present invention an amino acid is an L-amino acid, but in some embodiments it may be a D-amino acid. Alteration may therefore comprise modifying an L-amino acid into, or replacing it with, a D-amino acid. Methylated, acetylated and/or phosphorylated forms of amino acids are also known, and amino acids in the present invention may be subject to such modification.

Amino acid sequences in antibody domains and specific binding members of the invention may comprise non-natural or non-standard amino acids described above. In some embodiments non-standard amino acids (e.g. D-amino acids) may be incorporated into an amino acid sequence during synthesis, while in other embodiments the non-standard amino acids may be introduced by modification or replacement of the "original" standard amino acids after synthesis of the amino acid sequence.

Use of non-standard and/or non-naturally occurring amino acids increases structural and functional diversity, and can thus increase the potential for achieving desired NGF binding and neutralising properties in a specific binding member of the invention. Additionally, D-amino acids and analogues have been shown to have better pharmacokinetic profiles compared with standard L-amino acids, owing to *in vivo* degradation of polypeptides having L-amino acids after administration to an animal.

As noted above, a CDR amino acid sequence substantially as set out herein is preferably carried as a CDR in a human antibody variable domain or a substantial portion thereof. The HCDR3 sequences substantially as set out herein represent preferred embodiments of the present invention and it is preferred that each of these is carried as a HCDR3 in a human heavy chain variable domain or a substantial portion thereof.

Variable domains employed in the invention may be obtained or derived from any germ-line or rearranged human variable domain, or may be a synthetic variable domain based on consensus or actual sequences of known human variable domains. A CDR sequence of the invention (e.g. CDR3) may be introduced into a repertoire of variable domains lacking a CDR (e.g. CDR3), using recombinant DNA technology.

For example, Marks *et al.* (1992) describe methods of producing repertoires of antibody variable domains in which consensus primers directed at or adjacent to the 5' end of the variable domain area are used in conjunction with consensus primers to the third framework region of human VH genes to provide a repertoire of VH variable domains lacking a CDR3. Marks *et al.* further describe how this repertoire may be combined with a CDR3 of a particular antibody. Using analogous techniques, the CDR3-derived sequences of the present invention may be shuffled with repertoires of VH or VL domains lacking a CDR3, and the shuffled complete VH or VL domains combined with a cognate VL or VH domain to provide specific binding members of the

invention. The repertoire may then be displayed in a suitable host system such as the phage display system of WO92/01047 or any of a subsequent large body of literature, including Kay, Winter & McCafferty (1996), so that suitable specific binding members may be selected. A repertoire may consist of from anything from 10^4 individual members upwards, for example from 10^6 to 10^8 or 10^{10} members. Other suitable host systems include yeast display, bacterial display, T7 display, ribosome display and covalent display.

Analogous shuffling or combinatorial techniques are also disclosed by Stemmer (1994), who describes the technique in relation to a β-lactamase gene but observes that the approach may be used for the generation of antibodies.

A further alternative is to generate novel VH or VL regions carrying CDR-derived sequences of the invention using random mutagenesis of one or more selected VH and/or VL genes to generate mutations within the entire variable domain. Such a technique is described by Gram *et al.* (1992), who used error-prone PCR. In preferred embodiments one or two amino acid substitutions are made within a set of HCDRs and/or LCDRs.

Another method that may be used is to direct mutagenesis to CDR regions of VH or VL genes. Such techniques are disclosed by Barbas *et al.* (1994) and Schier *et al.* (1996).

All the above-described techniques are known as such in the art and the skilled person will be able to use such techniques to provide specific binding members of the invention using routine methodology in the art.

A further aspect of the invention provides a method for obtaining an antibody antigen-binding site specific for NGF antigen, the method comprising providing by way of addition, deletion, substitution or insertion of one or more amino acids in the amino acid sequence of a

VH domain set out herein a VH domain which is an amino acid sequence variant of the VH domain, optionally combining the VH domain thus provided with one or more VL domains, and testing the VH domain or VH/VL combination or combinations to identify a specific binding member or an antibody antigen-binding site specific for NGF antigen and optionally with one or more preferred properties, preferably ability to neutralise NGF activity. Said VL domain may have an amino acid sequence which is substantially as set out herein.

An analogous method may be employed in which one or more sequence variants of a VL domain disclosed herein are combined with one or more VH domains.

In a preferred embodiment, 1252A5 VH domain (SEQ ID NO: 392) may be subject to mutation to provide one or more VH domain amino acid sequence variants, optionally combined with 1252A5 VL (SEQ ID NO: 397).

A further aspect of the invention provides a method of preparing a specific binding member specific for NGF antigen, which method comprises:

(a) providing a starting repertoire of nucleic acids encoding a VH domain which either include a CDR3 to be replaced or lack a CDR3 encoding region;
(b) combining said repertoire with a donor nucleic acid encoding an amino acid sequence substantially as set out herein for a VH CDR3 such that said donor nucleic acid is inserted into the CDR3 region in the repertoire, so as to provide a product repertoire of nucleic acids encoding a VH domain;
(c) expressing the nucleic acids of said product repertoire;
(d) selecting a specific binding member specific for NGF; and
(e) recovering said specific binding member or nucleic acid encoding it.

Again, an analogous method may be employed in which a VL CDR3 of the invention is combined with a repertoire of nucleic acids encoding a VL domain that either include a CDR3 to be replaced or lack a CDR3 encoding region.

Similarly, one or more, or all three CDRs may be grafted into a repertoire of VH or VL domains that are then screened for a specific binding member or specific binding members specific for NGF.

In a preferred embodiment, one or more of 1252A5 HCDR1 (SEQ ID NO: 393), HCDR2 (SEQ ID NO: 394) and HCDR3 (SEQ ID NO: 395), or the 1252A5 set of HCDRs, may be employed, and/or one or more of 1252A5 LCDR1 (SEQ ID NO: 398), LCDR2 (SEQ ID NO: 399) and LCDR3 (SEQ ID NO: 400) or the 1252A5 set of LCDRs may be employed.

In other analogous embodiments 1152H5, 1165D4 or 1230H7 is substituted for 1252A5.

Similarly, other VH and VL domains, sets of CDRs and sets of HCDRs and/or sets of LCDRs disclosed herein may be employed.

A substantial portion of an immunoglobulin variable domain will comprise at least the three CDR regions, together with their intervening framework regions. Preferably, the portion will also include at least about 50% of either or both of the first and fourth framework regions, the 50% being the C-terminal 50% of the first framework region and the N-terminal 50% of the fourth framework region. Additional residues at the N-terminal or C-terminal end of the substantial part of the variable domain may be those not normally associated with naturally occurring variable domain regions. For example, construction of specific binding members of the present invention made by recombinant DNA techniques may result in the introduction of N- or C-terminal residues encoded by linkers introduced to facilitate cloning or other manipulation steps. Other manipulation steps include the introduction of linkers to join

variable domains of the invention to further protein sequences including antibody constant regions, other variable domains (for example in the production of diabodies) or detectable/functional labels as discussed in more detail elsewhere herein.

Although in a preferred aspect of the invention specific binding members comprising a pair of VH and VL domains are preferred, single binding domains based on either VH or VL domain sequences form further aspects of the invention. It is known that single immunoglobulin domains, especially VH domains, are capable of binding target antigens in a specific manner. For example, see the discussion of dAbs above.

In the case of either of the single specific binding domains, these domains may be used to screen for complementary domains capable of forming a two-domain specific binding member able to bind NGF.

This may be achieved by phage display screening methods using the so-called hierarchical dual combinatorial approach as disclosed in WO92/01047, in which an individual colony containing either an H or L chain clone is used to infect a complete library of clones encoding the other chain (L or H) and the resulting two-chain specific binding member is selected in accordance with phage display techniques such as those described in that reference. This technique is also disclosed in Marks *et al, ibid.*

Specific binding members of the present invention may further comprise antibody constant regions or parts thereof, preferably human antibody constant regions or parts thereof. For example, a VL domain may be attached at its C-terminal end to antibody light chain constant domains including human Cκ or Cλ chains, preferably Cλ chains. Similarly, a specific binding member based on a VH domain may be attached at its C-terminal end to all or part (e.g. a CH1 domain) of an immunoglobulin heavy chain derived from any antibody isotype, e.g. IgG, IgA, IgE and IgM and any of the isotype sub-classes, particularly

IgG1 and IgG4. IgG4 is preferred. IgG4 is preferred because it does not bind complement and does not create effector functions. Any synthetic or other constant region variant that has these properties and stabilizes variable regions is also preferred for use in embodiments of the present invention.

Specific binding members of the invention may be labelled with a detectable or functional label. Detectable labels include radiolabels such as ^{131}I or ^{99}Tc, which may be attached to antibodies of the invention using conventional chemistry known in the art of antibody imaging. Labels also include enzyme labels such as horseradish peroxidase. Labels further include chemical moieties such as biotin that may be detected via binding to a specific cognate detectable moiety, e.g. labelled avidin.

Specific binding members of the present invention are designed to be used in methods of diagnosis or treatment in human or animal subjects, preferably human.

Accordingly, further aspects of the invention provide methods of treatment comprising administration of a specific binding member as provided, pharmaceutical compositions comprising such a specific binding member, and use of such a specific binding member in the manufacture of a medicament for administration, for example in a method of making a medicament or pharmaceutical composition comprising formulating the specific binding member with a pharmaceutically acceptable excipient.

Clinical indications in which an anti-NGF antibody may be used to provide therapeutic benefit include pain, asthma, chronic obstructive pulmonary disease, pulmonary fibrosis, other diseases of airway inflammation, diabetic neuropathy, arthritis, psoriasis, cardiac arrhythmias, HIV and cancer. As already explained, anti-NGF treatment is indicated for all these diseases.

Anti-NGF treatment may be given orally, by injection (for example, subcutaneously, intravenously, intraperitoneal or intramuscularly), by inhalation, by the intravesicular route (instillation into the urinary bladder), or topically (for example intraocular, intranasal, rectal, into wounds, on skin). The route of administration can be determined by the physicochemical characteristics of the treatment, by special considerations for the disease or by the requirement to optimise efficacy or to minimise side-effects.

It is envisaged that anti-NGF treatment will not be restricted to use in the clinic. Therefore, subcutaneous injection using a needle free device is also preferred.

Combination treatments may be used to provide significant synergistic effects, particularly the combination of an anti-NGF specific binding member with one or more other drugs. A specific binding member according to the present invention may be provided in combination or addition to short or long acting analgesic, anti-inflammatory, anti-allergic, anti-asthmatic, anti-fibrotic, antiviral, chemotherapeutic agents and immunotherapeutic agents.

Combination treatment with one or more short or long acting analgesics and/or anti-inflammatory agents, such as opioids and non-steroid anti-inflammatory drugs (NSAIDs), may be employed for treatment of conditions characterized by pain and/or inflammation for example rheumatoid arthritis or post-surgical pain. Antibodies of the present invention can also be used in combination with anti-asthma, anti-allergic, or anti-fibrotic therapies, such as inhaled beta adrenoceptor agonists, steroids, cytokine antagonists, or other novel therapeutic approaches for treatment of asthma, allergic asthma, other allergic conditions, or any condition characterized by abnormal fibrosis. Antibodies of the present invention may also be used in combination with anti-infective agents, for example antiviral agents for the treatment of HIV infection.

In accordance with the present invention, compositions provided may be administered to individuals. Administration is preferably in a "therapeutically effective amount", this being sufficient to show benefit to a patient. Such benefit may be at least amelioration of at least one symptom. The actual amount administered, and rate and time-course of administration, will depend on the nature and severity of what is being treated. Prescription of treatment, e.g. decisions on dosage etc, is within the responsibility of general practitioners and other medical doctors, and may depend on the severity of the symptoms and/or progression of a disease being treated. Appropriate doses of antibody are well known in the art; see Ledermann et al. (1991) and Bagshawe (1991). Specific dosages indicated herein, or in the Physician's Desk Reference (2003) as appropriate for the type of medicament being administered, may be used. A therapeutically effective amount or suitable dose of a specific binding member of the invention can be determined by comparing its *in vitro* activity and *in vivo* activity in an animal model. Methods for extrapolation of effective dosages in mice and other test animals to humans are known.

The precise dose will depend upon a number of factors, including whether the antibody is for diagnosis or for treatment, the size and location of the area to be treated, the precise nature of the antibody (e.g. whole antibody, fragment or diabody), and the nature of any detectable label or other molecule attached to the antibody. A typical antibody dose will be in the range 100μg to 1 g for systemic applications, and 1μg to 1mg for topical applications. Typically, the antibody will be a whole antibody, preferably the IgG4 isotype. This is a dose for a single treatment of an adult patient, which may be proportionally adjusted for children and infants, and also adjusted for other antibody formats in proportion to molecular weight. Treatments may be repeated at daily, twice-weekly, weekly or monthly intervals, at the discretion of the physician. In preferred embodiments of the present invention, treatment is periodic, and the period between administrations is about two weeks or more, preferably

about three weeks or more, more preferably about four weeks or more, or about once a month. In other preferred embodiments of the invention, treatment may be given before, and/or after surgery, and more preferably, may be administered or applied directly at the anatomical site of surgical treatment.

Specific binding members of the present invention will usually be administered in the form of a pharmaceutical composition, which may comprise at least one component in addition to the specific binding member.

Thus pharmaceutical compositions according to the present invention, and for use in accordance with the present invention, may comprise, in addition to active ingredient, a pharmaceutically acceptable excipient, carrier, buffer, stabiliser or other materials well known to those skilled in the art. Such materials should be non-toxic and should not interfere with the efficacy of the active ingredient. The precise nature of the carrier or other material will depend on the route of administration, which may be oral, or by injection, e.g. intravenous.

Pharmaceutical compositions for oral administration may be in tablet, capsule, powder, liquid or semi-solid form. A tablet may comprise a solid carrier such as gelatin or an adjuvant. Liquid pharmaceutical compositions generally comprise a liquid carrier such as water, petroleum, animal or vegetable oils, mineral oil or synthetic oil. Physiological saline solution, dextrose or other saccharide solution or glycols such as ethylene glycol, propylene glycol or polyethylene glycol may be included.

For intravenous injection, or injection at the site of affliction, the active ingredient will be in the form of a parenterally acceptable aqueous solution which is pyrogen-free and has suitable pH, isotonicity and stability. Those of relevant skill in the art are well able to prepare suitable solutions using, for example, isotonic

vehicles such as Sodium Chloride Injection, Ringer's Injection, Lactated Ringer's Injection. Preservatives, stabilisers, buffers, antioxidants and/or other additives may be included, as required.

A composition may be administered alone or in combination with other treatments, either simultaneously or sequentially dependent upon the condition to be treated.

Specific binding members of the present invention may be formulated in liquid, semi-solid or solid forms depending on the physicochemical properties of the molecule and the route of delivery. Formulations may include excipients, or combinations of excipients, for example: sugars, amino acids and surfactants. Liquid formulations may include a wide range of antibody concentrations and pH. Solid formulations may be produced by lyophilisation, spray drying, or drying by supercritical fluid technology, for example. Formulations of anti-NGF will depend upon the intended route of delivery: for example, formulations for pulmonary delivery may consist of particles with physical properties that ensure penetration into the deep lung upon inhalation; topical formulations may include viscosity modifying agents, which prolong the time that the drug is resident at the site of action. In certain embodiments, the specific binding member may be prepared with a carrier that will protect the specific binding member against rapid release, such as a controlled release formulation, including implants, transdermal patches, and microencapsulated delivery systems. Biodegradable, biocompatible polymers can be used, such as ethylene vinyl acetate, polyanhydrides, polyglycolic acid, collagen, polyorthoesters, and polylactic acid. Many methods for the preparation of such formulations are known to those skilled in the art. See, e.g., Robinson, 1978.

The present invention provides a method comprising causing or allowing binding of a specific binding member as provided herein to NGF. As noted, such binding may take place *in vivo*, e.g. following administration of a specific binding member, or nucleic acid encoding

a specific binding member, or it may take place *in vitro*, for example in ELISA, Western blotting, immunocytochemistry, immuno-precipitation, affinity chromatography, or cell based assays such as a TF-1 assay.

The amount of binding of specific binding member to NGF may be determined. Quantitation may be related to the amount of the antigen in a test sample, which may be of diagnostic interest.

A kit comprising a specific binding member or antibody molecule according to any aspect or embodiment of the present invention is also provided as an aspect of the present invention. In a kit of the invention, the specific binding member or antibody molecule may be labelled to allow its reactivity in a sample to be determined, e.g. as described further below. Components of a kit are generally sterile and in sealed vials or other containers. Kits may be employed in diagnostic analysis or other methods for which antibody molecules are useful. A kit may contain instructions for use of the components in a method, e.g. a method in accordance with the present invention. Ancillary materials to assist in or to enable performing such a method may be included within a kit of the invention.

The reactivities of antibodies in a sample may be determined by any appropriate means. Radioimmunoassay (RIA) is one possibility. Radioactive labelled antigen is mixed with unlabelled antigen (the test sample) and allowed to bind to the antibody. Bound antigen is physically separated from unbound antigen and the amount of radioactive antigen bound to the antibody determined. The more antigen there is in the test sample the less radioactive antigen will bind to the antibody. A competitive binding assay may also be used with non-radioactive antigen, using antigen or an analogue linked to a reporter molecule. The reporter molecule may be a fluorochrome, phosphor or laser dye with spectrally isolated absorption or emission characteristics. Suitable fluorochromes include fluorescein, rhodamine, phycoerythrin and Texas Red. Suitable chromogenic dyes include diaminobenzidine.

Other reporters include macromolecular colloidal particles or particulate material such as latex beads that are coloured, magnetic or paramagnetic, and biologically or chemically active agents that can directly or indirectly cause detectable signals to be visually observed, electronically detected or otherwise recorded. These molecules may be enzymes, which catalyse reactions that develop, or change colours or cause changes in electrical properties, for example. They may be molecularly excitable, such that electronic transitions between energy states result in characteristic spectral absorptions or emissions. They may include chemical entities used in conjunction with biosensors. Biotin/avidin or biotin/streptavidin and alkaline phosphatase detection systems may be employed.

The signals generated by individual antibody-reporter conjugates may be used to derive quantifiable absolute or relative data of the relevant antibody binding in samples (normal and test).

The present invention also provides the use of a specific binding member as above for measuring antigen levels in a competition assay, that is to say a method of measuring the level of antigen in a sample by employing a specific binding member as provided by the present invention in a competition assay. This may be where the physical separation of bound from unbound antigen is not required. Linking a reporter molecule to the specific binding member so that a physical or optical change occurs on binding is one possibility. The reporter molecule may directly or indirectly generate detectable, and preferably measurable, signals. The linkage of reporter molecules may be directly or indirectly, covalently, e.g. via a peptide bond or non-covalently. Linkage via a peptide bond may be as a result of recombinant expression of a gene fusion encoding antibody and reporter molecule.

The present invention also provides for measuring levels of antigen directly, by employing a specific binding member according to the invention for example in a biosensor system.

The mode of determining binding is not a feature of the present invention and those skilled in the art are able to choose a suitable mode according to their preference and general knowledge.

As noted, in various aspects and embodiments, the present invention extends to a specific binding member that competes for binding to NGF with any specific binding member defined herein, e.g. 1252A5 IgG4. Competition between binding members may be assayed easily *in vitro*, for example by tagging a specific reporter molecule to one binding member which can be detected in the presence of other untagged binding member(s), to enable identification of specific binding members which bind the same epitope or an overlapping epitope.

Competition may be determined for example using ELISA in which NGF is immobilised to a plate and a first tagged binding member along with one or more other untagged binding members is added to the plate. Presence of an untagged binding member that competes with the tagged binding member is observed by a decrease in the signal emitted by the tagged binding member.

In testing for competition a peptide fragment of the antigen may be employed, especially a peptide including or consisting essentially of an epitope of interest. A peptide having the epitope sequence plus one or more amino acids at either end may be used. Specific binding members according to the present invention may be such that their binding for antigen is inhibited by a peptide with or including the sequence given. In testing for this, a peptide with either sequence plus one or more amino acids may be used.

Specific binding members that bind a specific peptide may be isolated for example from a phage display library by panning with the peptide(s).

The present invention further provides an isolated nucleic acid encoding a specific binding member of the present invention. Nucleic acid may include DNA and/or RNA. In a preferred aspect, the present invention provides a nucleic acid that codes for a CDR or set of CDRs or VH domain or VL domain or antibody antigen-binding site or antibody molecule, e.g. scFv or IgG4, of the invention as defined above.

The present invention also provides constructs in the form of plasmids, vectors, transcription or expression cassettes which comprise at least one polynucleotide as above.

The present invention also provides a recombinant host cell that comprises one or more constructs as above. A nucleic acid encoding any CDR or set of CDRs or VH domain or VL domain or antibody antigen-binding site or antibody molecule, e.g. scFv or IgG4 as provided, itself forms an aspect of the present invention, as does a method of production of the encoded product, which method comprises expression from encoding nucleic acid therefor. Expression may conveniently be achieved by culturing under appropriate conditions recombinant host cells containing the nucleic acid. Following production by expression a VH or VL domain, or specific binding member may be isolated and/or purified using any suitable technique, then used as appropriate.

Specific binding members, VH and/or VL domains, and encoding nucleic acid molecules and vectors according to the present invention may be provided isolated and/or purified, e.g. from their natural environment, in substantially pure or homogeneous form, or, in the case of nucleic acid, free or substantially free of nucleic acid or genes of origin other than the sequence encoding a polypeptide with the required function. Nucleic acid according to the present invention may comprise DNA or RNA and may be wholly or partially

synthetic. Reference to a nucleotide sequence as set out herein encompasses a DNA molecule with the specified sequence, and encompasses a RNA molecule with the specified sequence in which U is substituted for T, unless context requires otherwise.

Systems for cloning and expression of a polypeptide in a variety of different host cells are well known. Suitable host cells include bacteria, mammalian cells, plant cells, yeast and baculovirus systems and transgenic plants and animals. The expression of antibodies and antibody fragments in prokaryotic cells is well established in the art. For a review, see for example Plückthun (1991). A common, preferred bacterial host is *E. coli*.

Expression in eukaryotic cells in culture is also available to those skilled in the art as an option for production of a specific binding member for example Chadd & Chamow (2001), Andersen & Krummen (2002), Larrick & Thomas (2001). Mammalian cell lines available in the art for expression of a heterologous polypeptide include Chinese hamster ovary (CHO) cells, HeLa cells, baby hamster kidney cells, NS0 mouse melanoma cells, YB2/0 rat myeloma cells, human embryonic kidney cells, human embryonic retina cells and many others.

Suitable vectors can be chosen or constructed, containing appropriate regulatory sequences, including promoter sequences, terminator sequences, polyadenylation sequences, enhancer sequences, marker genes and other sequences as appropriate. Vectors may be plasmids e.g. phagemid, or viral e.g. 'phage, as appropriate. For further details see, for example, Sambrook & Russell (2001). Many known techniques and protocols for manipulation of nucleic acid, for example in preparation of nucleic acid constructs, mutagenesis, sequencing, introduction of DNA into cells and gene expression, and analysis of proteins, are described in detail in Ausubel et al., 1988 and Ausubel et al., 1999.

Thus, a further aspect of the present invention provides a host cell containing nucleic acid as disclosed herein. Such a host cell may be *in vitro* and may be in culture. Such a host cell may be *in vivo*. *In vivo* presence of the host cell may allow intracellular expression of the specific binding members of the present invention as "intrabodies" or intracellular antibodies. Intrabodies may be used for gene therapy.

A still further aspect provides a method comprising introducing such nucleic acid into a host cell. The introduction may employ any available technique. For eukaryotic cells, suitable techniques may include calcium phosphate transfection, DEAE-Dextran, electroporation, liposome-mediated transfection and transduction using retrovirus or other virus, e.g. vaccinia or, for insect cells, baculovirus. Introducing nucleic acid in the host cell, in particular a eukaryotic cell may use a viral or a plasmid based system. The plasmid system may be maintained episomally or may incorporated into the host cell or into an artificial chromosome. Incorporation may be either by random or targeted integration of one or more copies at single or multiple loci. For bacterial cells, suitable techniques may include calcium chloride transformation, electroporation and transfection using bacteriophage.

The introduction may be followed by causing or allowing expression from the nucleic acid, e.g. by culturing host cells under conditions for expression of the gene.

In one embodiment, the nucleic acid of the invention is integrated into the genome (e.g. chromosome) of the host cell. Integration may be promoted by inclusion of sequences that promote recombination with the genome, in accordance with standard techniques.

The present invention also provides a method that comprises using a construct as stated above in an expression system in order to express a specific binding member or polypeptide as above.

Aspects and embodiments of the present invention will now be illustrated, by way of example, with reference to the following experimentation and the accompanying drawings, in which:

Figure 1 shows concentration-inhibition curves for antibody neutralisation of human (Figure 1A) and rat (Figure 1B) NGF in the human TrkA receptor calcium mobilisation assay. Human IgG4 NGF antibodies were compared with the commercial NGF antibodies G1131, Mab5260Z, and MAB256 for inhibition of intracellular calcium mobilisation evoked by 1nM NGF. Data points indicate results from a single experiment and are mean ± sd of triplicate determinations for each antibody concentration.

Figure 2 shows inhibition of intracellular calcium mobilisation in HEK-293 cells recombinantly expressing the human TrkA receptor. Potency-optimised human IgG4 antibodies were evaluated for inhibition of responses evoked by 1nM human (Figure 2A), rat (Figure 2B), and mouse (Figure 2C) NGF. Data points indicate results from a single experiment and are mean ± sd of triplicate determinations for each antibody concentration.

Figure 3 shows the inhibitory effect of human IgG4 NGF antibodies in the PC12 cell survival assay. Cell survival was maintained by the presence of 1nM human NGF (Figure 3A) or rat NGF (Figure 3B). Data indicate mean ± sd for triplicate determinations from a single experiment.

Figure 4 shows inhibition of NGF-mediated TF-1 cell proliferation by germline and non-germline human IgG4 NGF antibodies, and the reference NGF antibody, MAB256. Cells were stimulated with 200pM human NGF (Figure 4A), rat NGF (Figure 4B), or mouse NGF (Figure 4C). Data represent the mean ± sem of triplicate determinations from a single experiment.

Figure 5 shows the lack of cross-reactivity of the human IgG4 NGF antibody 1252A5 with the neurotrophins BDNF, NT-3 and NT-4. 100ng neurotrophin was adsorbed per well. Each data point represents the mean ± sd of triplicate determinations from a single experiment.

Figure 6 shows saturation binding curves for human ^{125}I-NGF binding to the human IgG4 NGF antibodies 1252A5 (Figure 6A) and G1152H5 (Figure 6B). Calculated Kd values are 0.35nM and 0.37nM, respectively, and are the result of a single experiment.

Figure 7 shows saturation binding curves for rat ^{125}I-NGF binding to the human IgG4 NGF antibodies 1252A5 (Figure 7A) and G1152H5 (Figure 7B). Calculated Kd values are 0.44nM and 0.50nM, respectively, and are the result of a single experiment.

Figure 8 shows concentration-dependent inhibition of human (Figure 8A) or rat (Figure 8B) ^{125}I-NGF binding to TrkA receptor fusion protein, by human IgG4 or reference antibodies. The concentration of radiolabelled NGF in each assay well was approximately 150pM. Data indicate the result of a single experiment. See also Tables 6 and 7.

Figure 9 shows concentration-dependant inhibition of human (Figure 9A) or rat (Figure 9B) ^{125}I-NGF binding to p75 receptor fusion protein, by human IgG4 or reference antibodies. The concentration of radiolabelled NGF in each assay well was approximately 150pM. Data indicate the result of a single experiment. See also Tables 8 and 9.

Figure 10 shows dose-related inhibition of carrageenan-induced thermal hyperalgesia in the mouse, 48h after systemic administration of the human IgG4 anti-NGF, 1252A5.

EXAMPLE 1
Isolation of anti-NGF scFv

ScFv antibody repertoire
Three large single chain Fv (scFv) human antibody libraries cloned into a phagemid vector, were used for selections. The libraries were derived from (A) spleen lymphocytes (Hutchings, 2001), (B) a combination of peripheral blood lymphocytes, tonsil B cells and bone marrow B cells (Vaughan et al., 1996) and (C) the light chains and VH CDR3 regions of A combined with the framework of the DP47 germline heavy chain.

Selection of scFv
The phage selection procedure used was essentially as described in Hutchings, *supra*. ScFv that recognised NGF were isolated from phage display libraries in a series of selection cycles on human and rat NGF. In brief, unmodified antigen was coated to Nunc Maxisorb tubes. Phage were incubated on the antigen in a total volume of 500μl for 1h prior to washing to remove unbound phage. Bound phage were then rescued as described by Vaughan *et al., supra* and the selection process repeated. To assist the isolation of human/rodent cross-reactive scFv alternate rounds of selection were performed on the respective species isoforms of NGF. A maximum of four rounds of selection was performed with any one library using this alternating isoform selection strategy. Either human or rat β-NGF were used as the initial antigen for first round selections.

Outputs from rounds 2-4 were prioritised for biochemical screening based on the percentage of NGF-specific clones isolated and the sequence diversity of these clones. The percentage of NGF-specific clones was determined in each case by phage ELISA. Sequence diversity was determined by DNA sequencing.

Phage ELISA protocol
Cultures of phage transduced bacteria were prepared in 1ml 2xTY medium containing 100μg/ml ampicillin and 50μg/ml kanamycin with shaking at

30°C for 16h. Phage supernatant was prepared by centrifugation of the culture (10 min at 3000rpm) and blocking with 3% w/v milk powder in PBS for 1h. Blocked phage were then added to plates previously coated with (1μg/ml) antigen or irrelevant antigen. Plates were washed between each step with three rinses in PBS-Tween 20 (0.1% v/v) followed by three rinses in PBS. Bound phage were detected by incubation with horseradish peroxidase (HRP)/anti-M13 conjugate (Amersham UK) diluted 1:5000 in 3% w/v milk powder PBS for 1h, and developed by incubation with tetramethylbenzidine (TMB) substrate (Sigma). The colorimetric reaction was stopped after an appropriate period by adding 0.5M sulphuric acid. Absorbance readings were taken at 450nm. Clones which bound specifically to the antigen were identified as having a signal on the antigen greater than or equal to 5x that on the irrelevant antigen.

DNA Sequencing

Double stranded DNA template for sequencing was obtained by PCR of scFv using the primers FDTETSEQ24 (TTTGTCGTCTTTCCAGACGTTAGT - SEQ ID NO: 534) and PUCreverse (AGCGGATAACAATTTCACACAGG - SEQ ID NO: 535). Excess primer and dNTPs from the primary PCR were removed using Macherey-Nagel Nucleofast 96 well PCR plates (Millipore) according to the manufacturer's recommendations. VH genes were sequenced with primer Lseq (GATTACGCCAAGCTTTGGAGC SEQ ID NO: 536). VL genes were sequenced with primer MYC Seq 10 (CTCTTCTGAGATGAGTTTTTG SEQ ID NO: 537). Each reaction mixture contained 20-40ng DNA, 3 - 20pmol primer and 4μl Big Dye Terminator V3.0 (Applied Biosystems, UK) in a volume of 20μl. Sequencing reactions consisted of 25 cycles of 96°C 10s; 50°C, 5s; 60°C, 4min. Samples were run and analysed on an Applied Biosystems 3700 DNA Analyser. Areas of ambiguity were analysed manually using Continuity software developed in-house (Cambridge Antibody Technology, UK) and SeqEd DNA sequence manipulation software (Applied Biosystems, UK).

Biochemical screen for NGF-neutralising scFv

The output from the phage selection process was further screened to identify clones that inhibited NGF binding to a TrkA receptor extracellular domain fusion protein.

Crude scFv samples were prepared from periplasmic lysates of *E.coli* TG-1 bacteria transfected with selected phage for evaluation in the binding assay. Nunc Maxisorb 96 well plates were coated overnight with human TrkA receptor extracellular domain fusion protein (R&D Systems; coating concentration; human NGF assay, 0.25nM; rat NGF assay, 1nM). Assay plates were washed with PBS / Tween 20, blocked for 2h using 1% bovine serum albumin (BSA) in PBS, and washed again. ScFv samples were preincubated for 30min with 1nM human or rat recombinant β-NGF (R&D Systems) in 1% BSA. Samples were transferred in a volume of 100µl to assay plates and incubated for 60min at room temperature. Plates were washed and NGF that remained bound to the plates was labelled using 0.3µg/ml of either anti-human NGF biotin (Peprotech) or anti-rat NGF biotin (R&D Systems) diluted in 1% BSA, followed by incubation for 60min at room temperature. Biotin-labelled anti-NGF was detected using the DELFIA (Wallac) time-resolved fluorescence detection system. Briefly, plates were washed and 100µl streptavidin Eu^{3+} added to each well, diluted 1/1000 in DELFIA assay buffer. Plates were incubated for a further 60 min at room temperature and washed with DELFIA wash buffer, followed by addition of 100µl DELFIA enhancement solution to each well. Plates were read using a Wallac Victor fluorimetric plate reader (excitation wavelength 314nm; emission wavelength 615nm).

Clones that inhibited both human and rat NGF binding by more than 70% as periplasmic lysate scFv preparations were re-evaluated as purified scFv in the binding assay. Purified scFv preparations were prepared as described in Example 3 of WO01/66754. Protein concentrations of purified scFv preparations were determined using the BCA method (Pierce). Re-assay highlighted the following scFv antibodies that were

potent neutralisers of human and rat NGF binding to the human TrkA receptor extracellular domain fusion protein:

1064F8 (VH SEQ ID NO: 2; VL SEQ ID NO: 7),
1022E3 (VH SEQ ID NO: 12; VL SEQ ID NO: 17),
1083H4 (VH SEQ ID NO: 22; VL SEQ ID NO: 27),
1021E5 (VH SEQ ID NO: 32; VL SEQ ID NO: 37),
1033G9 (VH SEQ ID NO: 42; VL SEQ ID NO: 47),
1016A8 (VH SEQ ID NO: 52; VL SEQ ID NO: 57),
1028F8 (VH SEQ ID NO: 62; VL SEQ ID NO: 67),
1033B2 (VH SEQ ID NO: 72; VL SEQ ID NO: 77),
1024C4 (VH SEQ ID NO: 82; VL SEQ ID NO: 87), and
1057F11 (VH SEQ ID NO: 92; VL SEQ ID NO: 97).

EXAMPLE 2

Expression of human IgG4 antibodies and in vitro functional evaluation of NGF-neutralising potency

The NGF-neutralising scFv 1064F8, 1022E3, 1083H4, 1021E5, 1033G9, 1016A8, 1028F8, 1033B2, 1024C4, and 1057F11 were reformatted as human IgG4 antibodies and assayed for NGF neutralising potency in a whole-cell assay system.

IgG conversion

Vectors were constructed for the most potent scFv clones to allow re-expression as whole antibody human IgG4, essentially as described by Persic *et al.* (1997). EBNA-293 cells maintained in conditioned medium were co-transfected with constructs expressing heavy and light chain domains. Whole antibody was purified from the medium using protein A affinity chromatography (Amersham Pharmacia). The purified antibody preparations were sterile filtered and stored at 4°C in phosphate buffered saline (PBS) prior to *in vitro* potency evaluation. Protein concentration was determined spetrophotometrically according to Mach *et al.* (1992).

FLIPR assay of intracellular calcium mobilisation

The potency and efficacy of anti-human IgGs for neutralising NGF were determined in a cell-based fluorescent calcium-mobilisation assay. The potency of human antibodies was compared with mouse anti-human NGF (MAB256; R&D Systems), rat anti-mouse NGF (G1131; Promega), and mouse anti-mouse NGF (MAB5260Z; Chemicon). Anti-NGF IgGs were co-incubated with recombinant human β-NGF (Calbiochem, 480275) or recombinant rat β-NGF (R&D Systems, 556-NG-100). The complex was then added to HEK293 cells expressing recombinant human TrkA receptors loaded with the calcium sensitive dye, Fluo-4, and then Ca^{2+}-dependent fluorescence was monitored.

HEK cells (peak-S, Edge Biosystems) transfected with recombinant human TrkA (obtained from M. Chao, Skirball Institute, NY) were grown in Dulbecco's Modified Eagle's Medium (MEM, Cellgro, MT10-017-CV) supplemented with 10% fetal bovine serum (Hyclone, SH30071.03), 1.5 μg/ml puromycin (Edge Biosystems, 80018) and 1% penicillin-streptomycin. Confluent cells were harvested by dislodging the cells with Dulbecco's phosphate buffered saline (DPBS), and then loaded with loading buffer containing 6 μM Fluo-4 (Molecular Probes) at 37°C for 1.5h in the presence of an anion transport inhibitor (2.5 mM probenecid in 1% FBS/MEM). After washing the cells once with assay buffer (2.5 mM probenecid in 0.1% BSA Hank's / HEPES), the cells were plated on poly-D-lysine coated, clear bottom 96-well plates (Costar #3904) at approximately 60,000 cells/well in 120 μl. The cells were incubated in the dark for 30 min at room temperature, and the plates were then centrifuged at 1,200 rpm (290 x g) for 5min. Prior to testing, the plates were pre-warmed at 35°C for 20 min. Test IgGs were assayed at 7 concentrations in triplicate wells. Thirty-five microlitres of 10X anti-NGF IgG and equal amounts of 10 nM human-, rat- or mouse 2.5S NGF were pre-incubated at room temperature for approximately 1h. Plates containing the pre-complexed IgGs and plates containing 100 μl/well assay buffer were pre-warmed at 35°C for 20min before testing on the Fluorometric Imaging Plate Reader (FLIPR;

Molecular Devices). Following the addition of 80µl/well assay buffer to the cell plate and incubation for 5 min at 35°C, 50µl/well of diluted anti-human IgG / NGF complex was added to the cell plate in the FLIPR with continuous monitoring of the Ca^{2+}-dependent fluorescence. The NGF-induced fluorescence was calculated as the difference between the baseline fluorescence intensity just prior to NGF addition and highest fluorescence intensity attained in 2 minutes following NGF addition. The average of triplicate NGF-induced fluorescence values in the absence of antibody was defined as 100% calcium mobilisation. In the presence of antibody, the average ± SD of triplicate NGF-induced fluorescence values was calculated as a percent of control calcium mobilisation. The percent of control calcium mobilisation values were plotted as function of the log of the IgG concentration. IC_{50} values were calculated by fitting the sigmoidal dose-response (variable slope) function using Prism (GraphPad).

All antibodies tested displayed concentration-related inhibition of human NGF-evoked intracellular calcium mobilisation. The rank order of potency for neutralisation of human NGF was 1064F8 > 1022E3 > 1083H4 ≥ 1033G9 ≥ 1016A8 ≥ 1028F8 ≥ 1021E5 ≥ 1033B2 >> 1024C4 >> 1057F11 (Table 1). Five antibodies were further evaluated for functional neutralisation of rat NGF, and these were approximately equipotent against both species isoforms (Figures 1A and 1B; Table 1).

EXAMPLE 3
Isolation of optimised human IgG4 NGF antibodies

Ribosome display scFv potency optimisation

Large ribosome display libraries were created and selected for scFv that specifically recognised recombinant human NGF (R&D Systems), essentially as described in Hanes *et al.* (2000). Initially, the clones 1064F8, 1022E3, 1083H4, 1021E5, 1033G9, 1016A8, 1028F8, 1033B2, and 1024C4 were converted to ribosome display format, where the

templates were subsequently used for library creation. The clone 1057F11 was not chosen for potency optimisation, and therefore a ribosome display template was not made for this antibody. On the DNA level, a T7 promoter was added at the 5'-end for efficient transcription to mRNA. On the mRNA level, the construct contained a prokaryotic ribosome-binding site (Shine-Dalgarno sequence). At the 3'end of the single chain, the stop codon was removed and a portion of gIII was added to act as a spacer (Hanes *et al.*, supra).

Ribosome display libraries derived from 1064F8, 1022E3, 1083H4, 1021E5, 1033G9, 1016A8, 1028F8, 1033B2, and 1024C4 were created by mutagenesis of the scFv HCDR3. PCR reactions were performed with non-proof reading *Taq* polymerase. Affinity-based selections were performed whereby, following incubation with the library, biotinylated human NGF was coupled to streptavidin-coated paramagnetic beads (Dynal M280). Bound tertiary complexes (mRNA-ribosome-scFv) were recovered by magnetic separation whilst unbound complexes were washed away. The mRNA encoding the bound scFv were then rescued by RT-PCR as described in Hanes *et al.*, (*supra*) and the selection process repeated with decreasing concentrations (100 nM - 10 pM over five rounds) of biotinylated human NGF present during the selection.

Error-prone PCR was also used to further increase library size. An error rate of 7.2 mutations per 1,000 bp was employed (Diversify™, Clontech) during the selection regime. Error-prone PCR reactions were performed before selections commenced at rounds three and four using biotinylated human NGF concentrations of 1 nM and 0.1 nM, respectively.

A representative proportion of scFv from the output of selection rounds three, four and five were ligated into pCantab6 vector (Vaughan *et al.*, 1996) and cloned in the TG1 strain of *E.coli*. A sample of these scFv was DNA sequenced as described in Example 1 to confirm sequence diversity of the output before screening *in vitro* for NGF neutralising activity. Clones were screened as unpurified scFv in the

NGF / TrkA receptor extracellular domain fusion protein binding assay, as described in Example 1. The concentration of the periplasmic lysate scFv preparations in the assays was reduced to 0.5% - 5% of the final assay volume, and clones that inhibited both human and rat NGF binding >95% were isolated for further study. In this way a panel of potency-optimised, cross-reactive NGF neutralisers was isolated. Surprisingly, the most potent NGF neutralisers were derived from the parent clones 1021E5 and 1083H4, which were not the most potent of the parent antibodies. Optimised clones were sequenced and reassayed as purified scFv to confirm potency before reformatting to human IgG4 as described in Example 2.

Antibodies derived from the parent clone 1021E5 (VH SEQ ID NO: 32; VL SEQ ID NO: 37) and converted to human IgG4 format were 1126F1 (VH SEQ ID NO: 102; VL SEQ ID NO: 107), 1126G5 (VH SEQ ID NO: 112; VL SEQ ID NO: 117), 1126H5 (VH SEQ ID NO: 122; VL SEQ ID NO: 127), 1127D9 (VH SEQ ID NO: 132; VL SEQ ID NO: 137), 1127F9 (VH SEQ ID NO: 142; VL SEQ ID NO: 147), 1131D7 (VH SEQ ID NO: 152; VL SEQ ID NO: 157), 1131H2 (VH SEQ ID NO: 162; VL SEQ ID NO: 167), 1132A9 (VH SEQ ID NO: 172; VL SEQ ID NO: 177), 1132H9 (VH SEQ ID NO: 182; VL SEQ ID NO: 187), 1133C11 (VH SEQ ID NO: 192; VL SEQ ID NO: 197), 1134D9 (VH SEQ ID NO: 202; VL SEQ ID NO: 207), 1145D1 (VH SEQ ID NO: 212; VL SEQ ID NO: 217), 1146D7 (VH SEQ ID NO: 222; VL SEQ ID NO: 227), 1147D2 (VH SEQ ID NO: 232; VL SEQ ID NO: 237), 1147G9 (VH SEQ ID NO: 242; VL SEQ ID NO: 247), 1150F1 (VH SEQ ID NO: 252; VL SEQ ID NO: 257), 1152H5 (VH SEQ ID NO: 262; VL SEQ ID NO: 267), 1155H1 (VH SEQ ID NO: 272; VL SEQ ID NO: 277), 1158A1 (VH SEQ ID NO: 282; VL SEQ ID NO: 287), 1160E3 (VH SEQ ID NO: 292; VL SEQ ID NO: 297), 1165D4 (VH SEQ ID NO: 302; VL SEQ ID NO: 307), 1175H8 (VH SEQ ID NO: 312; VL SEQ ID NO: 317), 1211G10 (VH SEQ ID NO: 322; VL SEQ ID NO: 327), 1214A1 (VH SEQ ID NO: 332; VL SEQ ID NO: 337), 1214D10 (VH SEQ ID NO: 342; VL SEQ ID NO: 347), 1218H5 (VH SEQ ID NO: 352; VL SEQ ID NO: 357),and 1230H7 (VH SEQ ID NO: 362; VL SEQ ID NO: 367).

Antibodies derived from the parent clone 1083H4 (VH SEQ ID NO: 22; VL SEQ ID NO: 27) and converted to human IgG4 format were 1227H8 (VH SEQ ID NO: 372; VL SEQ ID NO: 377) and 1230D8 (VH SEQ ID NO: 382; VL SEQ ID NO: 387).

Germlining framework regions of 1133C11 to derive 1252A5 and other 1021E5 variants

Examination of the VH and VL CDR sequence information for optimised clones derived from 1021E5 highlighted that a large proportion of these antibodies contained the amino acid sequence LNPSLTA (SEQ ID NO: 531) in VH CDR3 (i.e. amino acids 100A to 100G according to the Kabat numbering system). These clones are shown in Table 2a, highlighting how they vary in amino acid sequence in the VH and VL CDR regions, together with an estimate of their NGF neutralising potency when assayed as purified scFv. Of these clones, 1133C11 differed in CDR regions from 1021E5 only by the 7 consecutive amino acids 100A to 100G as described. Therefore, 1133C11 was chosen for germlining, first to confirm that potency was retained with the modified framework, and second to allow subsequent CDR mutations to be introduced, if desired, in order to generate further germline antibodies of interest from the same lineage.

The derived amino acid VH and VL sequences of 1133C11 were aligned to the known human germline sequences in the VBASE database (Tomlinson [1997], MRC Centre for Protein Engineering, Cambridge, UK) and the closest germline identified. The closest germline for the VH of 1133C11 was identified as DP10, a member of the VH1 family. The 1133C11 VH has 5 amino acid changes from the DP10 germline within framework regions. The closest germline for the VL of 1133C11 was identified as DPL5, a member of the Vλ1 family. The 1133C11 VL has only 4 changes from the germline within framework regions. Framework regions of 1133C11 were returned to germline by site directed mutagenesis of the scFv to derive the scFv 1252A5 (VH SEQ ID NO: 392; VL SEQ ID NO: 397). This was converted to human IgG4 as described in

Example 2. Germlining of other variants of 1021E5-derived clones was achieved by introducing CDR mutations onto the germlined 1252A5 IgG4 backbone. This method resulted in generation of the germlined antibodies G1152H5 (VH SEQ ID NO: 402; VL SEQ ID NO: 407), G1165D4 (VH SEQ ID NO: 412; VL SEQ ID NO: 417) and G1230H7 (VH SEQ ID NO: 422; VL SEQ ID NO: 427).

EXAMPLE 4

Evaluation of optimised human IgG4 antibodies in the FLIPR assay of intracellular calcium mobilisation

Optimised human IgG4 NGF antibodies were evaluated in an assay of NGF-evoked intracellular calcium mobilisation in cells recombinantly expressing the human TrkA receptor, as described in Example 2. Antibodies were assayed for neutralising activity against human, rat, and mouse NGF (Figures 2A, 2B, and 2C; Table 3).

Intracellular calcium mobilisation evoked by 1nM NGF was inhibited by all optimised human antibodies tested. Optimised antibodies displayed subnanomolar IC_{50} values, in most cases representing greater than one hundredfold enhancement of NGF neutralising potency over the parent IgGs (Table 3). Neutralising potencies (IC_{50}) of the germlined human IgG4 antibodies against the human, rat and mouse NGF isoforms were, respectively:

1252A5 - 0.33nM, 0.29nM, and 0.26nM;
G1152H5 - 0.22nM, 0.27nM, and 0.18nM;
G1165D4 - 0.32nM, 0.33nM, and 0.27nM;
G1230H7 - 0.31nM, 0.34nM, and 0.25nM.

These results highlight the efficiency and value of the ribosome display technique for antibody potency optimisation. A more conventional approach to antibody optimisation in the past has been to generate phage display libraries of variant scFv antibodies. This process is labour intensive and slower than the ribosome display

method, which often means that only a single parent scFv is used as the starting point for library construction. The relative ease of generating ribosome display libraries allows multiple scFv parents to be optimised simultaneously and, as demonstrated in Example 3, this can lead to the isolation of highly potent antibodies derived from parent clones that would have been otherwise overlooked for optimisation.

EXAMPLE 5

Evaluation of optimised human IgG4 antibodies in a PC12 cell survival assay

In the PC12 assay, NGF maintains the survival of serum-deprived rat PC12 cells expressing native TrkA and p75 receptors for two days. Neutralising NGF antibodies reduce cell survival measured with AlamarBlue.

Rat pheochromocytoma PC12 cells were grown in RPMI 1640 (Cellgro, 18040181) supplemented with 5% fetal bovine serum (JRH,12103-78P), 10% heat-inactivated donor horse serum (JRH, 12446-77P), and 1% penicillin-streptomycin. The cells were harvested by trituration, and then washed twice with serum-free RPMI 1640 containing 0.01% BSA (Sigma, A7030). Cells were plated in rat tail collagen (Biological Technology Institute, BT-274)-coated 96 well plates at 50,000 cells/well in 120 µl serum-free media with 0.01% BSA. Serial dilutions of 5X anti-human NGF IgGs were made using serum-free media and 40 µl/well was added to the cell plate. 40µl/well 0.5 nM human β-NGF (Calbiochem, 480275) or rat recombinant NGF (R&D Systems, 556-NG-100) was added to the plate, and the total volume was brought up to 200 µl/well with serum-free medium. Maximal cell death was defined by 80 µl/well of serum-free medium in triplicate wells. 100% survival was defined by 40µl/well serum-free media and 40µl/well 0.5nM NGF in triplicate wells. The plates were incubated at 37^{0}C in 5% CO_2 for 48h.

To measure the cell viability, 22 μl/well AlamarBlue was added and the plates were read immediately to determine the background fluorescence in each well with a fluorometric plate reader (BMG) at 530 nm excitation wavelength and 590 nm emission wavelength. Following an incubation for 6~7h at 37^{0}C, the plate was re-read to determine total fluorescence. The Alamar blue fluorescence was calculated as the difference between the background and total fluorescence intensities. In the presence of antibody, the average $\pm$ SD of triplicate fluorescence values was calculated as a percent of the average of triplicate 100% survival fluorescence values. The percent of control survival values were plotted as function of the log of the IgG concentration. IC_{50} values were calculated by fitting the sigmoidal dose-response (variable slope) function using Prism (GraphPad).

Results from the PC12 assay further confirmed the increased potency of optimised human IgG4 NGF antibodies over their parent IgGs. Antibodies inhibited human and rat NGF-maintained PC12 cell survival in a concentration-related manner (Figures 3A and 3B; Table 3). Germlining appeared to reduce the NGF neutralising potency of the test antibodies, particularly against the rat NGF isoform. For example, the mean IC_{50} of the germline antibody G1152H5 for inhibition of cell survival mediated by 1nM human or rat NGF was 1.1nM and 7.3nM, respectively. In contrast, the mean IC_{50} for neutralisation of 1nM human or rat NGF by 1152H5 (ie non-germline antibody) was 0.40nM and 0.38nM, respectively.

EXAMPLE 6

NGF-neutralising activity in a TF-1 cell proliferation assay

The TF-1 cell line is a human premyeloid cell line that can be stimulated to proliferate by exogenous growth factors and cytokines. TF-1 cells express the human TrkA receptor, and proliferate in response to activation with NGF. The TF-1 cell proliferation assay was used to further characterise the in vitro functional potency of neutralising human IgG4 NGF antibodies.

TF-1 cells were obtained from R&D Systems and maintained according to supplied protocols. Assay media comprised of RPMI-1640 with GLUTAMAX I (Invitrogen) containing 5% foetal bovine serum (Hyclone) and 1% sodium pyruvate (Sigma). Prior to each assay, TF-1 cells were pelleted by centrifugation at 300 x *g* for 5 minutes, the media removed by aspiration and the cells resuspended in assay media. This process was repeated three times with cells resuspended at a final concentration of 10^5 /ml in assay media and 100µl was added to each well of a 96 well flat bottomed tissue culture assay plate to give final cell density at $1x10^4$/well. Test solutions of antibodies (in triplicate) were diluted to give a final assay concentration of 1µg/ml in assay media and titrated 1:5 across assay plate. An irrelevant antibody (CAT-001) not directed at NGF, was used as a negative control. In addition, a reference monoclonal antibody MAB256 (R&D Systems) was used as a positive control. Fifty microlitres of test antibodies were then added to each well followed by 50µl of native purified murine (7S form; Invitrogen), rat (Sigma) or human (Sigma) NGF diluted to give a final assay concentration of 200pM. Assay plates were incubated for 68h at 37°C in 5% CO_2 in a humidified chamber. Twenty microlitres of tritiated thymidine (5.0 µCi/ml, NEN) was then added to each assay well and assay plates were returned to the incubator for a further 5h. Cells were harvested onto 96 well glass fibre filter plates (Perkin Elmer) using a cell harvester. MicroScint 20TM (50µl) was then added to each well of the filter plate and [^{3}H]-thymidine incorporation quantified

using a Packard TopCount microplate liquid scintillation counter. In the presence of antibody, thymidine incorporation (quantified as counts per minute) was calculated as the difference between the average background (i.e. cells not exposed to NGF) and the average total (i.e. cells stimulated with NGF) counts per minute and expressed as a percent of maximum proliferation. The percent maximum proliferation was plotted as function of the log of the IgG concentration. IC_{50} values were calculated by fitting the sigmoidal dose (variable slope) function using GraphPad prism.

Human IgG4 NGF antibodies were potent inhibitors of TF-1 cell proliferation mediated by human, rat and mouse NGF isoforms(Figures 4A, 4B, and 4C). These results demonstrate that antibodies derived from the 1021E5 lineage can disrupt NGF signalling mediated by activation of native human NGF receptors in vitro. In accordance with the observed activity of human NGF antibodies in the PC12 cell survival assay (Example 5), non-germline antibodies were more potent than their germline counterparts in the TF-1 proliferation assay (Table 3). Based on mean IC50 data, the rank order of potency of the antibodies tested for inhibition of proliferation mediated by 200pM human NGF was 1133C11 > 1152H5 > 1252A5 = G1152H5 >> MAB256.

EXAMPLE 7
Cross-Reactivity of anti-NGF IgGs with other neurotrophins

ELISAs were performed to determine the cross-reactivity of the anti-NGF IgGs for other neurotrophins. The ELISAs consisted of coating plates with 100 ng/well human NGF (R&D systems, 256-GF), brain derived neurotrophic factor (BDNF; R&D systems, 248-BD), neurotrophin-3 (NT-3; R&D systems, 257-N3), or neurotrophin-4 (NT4; R&D systems, 257-N4) at room temperature for 5-6 h, followed by blocking the plates with 0.25% HSA at 4^0C overnight. Increasing concentrations of anti-NGF IgG, ranging from 0.03 - 10 nM, were incubated at room temperature for 2 h to allow binding to each neurotrophin. Anti-NGF IgGs were detected with a biotinylated anti-human polyclonal antibody (1:300) (Rockland

609-1602), streptavidin-linked alkaline phosphatase (1:1000), and fluorescent Substrate A. Positive controls demonstrating neurotrophin binding to the plate utilised commercial biotinylated anti-human polyclonal antibodies (R&D Systems anti-NGF BAF 256, anti-BDNF BAM 648, anti-NT-3 BAF 267, anti-NT-4 BAF 268), which were detected directly using streptavidin-linked alkaline phosphatase and subsequent addition of Substrate A. Nonspecific binding was determined using wells coated with BSA instead of neurotrophin. Product development was followed over time from 0 - 60 min after addition of Substrate A. Anti-NGF IgG 1064F8 was used to optimise the assay. For 1064F8, there was linear product development for 15 min, which then leveled off with time, probably due to substrate depletion. Cross-reactivities were calculated as a percent of the specific binding to neurotrophin relative to NGF for all concentrations of IgG. For high affinity IgGs such as 1064F8, percent cross-reactivities were calculated using 15 min product development data. For low affinity IgGs such as 1016A8, percent cross-reactivities were calculated using 60 min product development data.

The cross-reactivities of seven human IgG4 NGF antibodies to BDNF, NT-3, and NT-4 relative to NGF were determined. At the concentrations tested, all seven antibodies showed negligible cross-reactivity (Table 4). For example, with 1252A5 the highest levels of cross-reactivity observed with NT-3, NT-4 and BDNF were 1.1%, 0.9% and 1.4%, respectively (Figure 5).

EXAMPLE 8
Determination of the NGF-binding affinity of human NGF antibodies

The NGF-binding affinities of human IgG4 NGF antibodies were determined using a radioligand-binding assay format, performed at room temperature. Briefly, flashplates (Perkin Elmer SMP200) were coated with 100µl/well of 2.2µg/ml goat anti-human IgG (Sigma-Aldrich, UK) in phosphate buffered saline (PBS) for 1h. Wells were washed with PBS and then blocked for 1h with 200µl/well PBS containing 3% w/v bovine serum

albumin (BSA; Sigma-Aldrich, UK). Wells were washed with PBS and 10 ng of human NGF antibody was added to each well in a volume of 0.1ml PBS containing 0.5% w/v BSA. Following incubation for 1h plates were washed with PBS.

Radioiodinated human and rat NGF were obtained from Amersham UK (human ^{125}I-NGF, Amersham cat. no. IM286; rat ^{125}I-NGF, custom-labelled recombinant rat β-NGF purchased from R&D Systems, cat. no. 556-GF-100). Each ^{125}I-NGF isoform was serially diluted in assay buffer (PBS containing 0.5% w/v BSA and 0.05% v/v Tween 20) and duplicate 100µl samples were added to the assay plate to give a measure of 'total binding' over the concentration range 2pM-15nM. Non-specific binding (NSB) was determined at each ^{125}I-NGF concentration by measuring binding in the presence of a large excess of non-radiolabelled NGF. NSB wells contained ^{125}I-NGF (2pM-15nM) together with a final concentration of 500 nM unlabelled human β-NGF (R&D Systems Cat. No. 256-GF-100) or rat β-NGF (R&D Systems Cat. No. 556-GF-100), as appropriate. Plates were incubated overnight, and wells counted for 1min on a gamma counter (TopCount NXT, Perkin Elmer). Specific binding was calculated according to the formula 'specific binding = total binding - non-specific binding'. Binding curves were plotted and binding parameters determined according to a one-site saturation binding model using Prism software (GraphPad Software Inc., USA).

Human and rat ^{125}I-NGF displayed saturable, high affinity binding to the human IgG4 NGF antibodies, 1252A5 and G1152H5 (Figures 6 and 7). Calculated Kd values for the binding interaction with human ^{125}I-NGF were 0.35nM for 1252A5, and 0.37nM for G1152H5. Kd values for rat ^{125}I-NGF binding were 0.44nM for 1252A5 and 0.50nM for G1152H5.

EXAMPLE 9

Determination of Ki values for inhibition of NGF binding to human TrkA and p75 receptors

Experiments were performed to determine whether the antibodies 1252A5 and G1152H5 display differential inhibition of NGF binding to TrkA and p75 receptors. Competition binding experiments were designed in order to calculate binding inhibition constant (Ki) values. IC_{50}s were calculated for antibody-mediated inhibition of radiolabelled human or rat NGF binding to human TrkA- or p75-receptor fusion proteins. Ki values were then derived using the Cheng-Prusoff equation.

Human TrkA and p75 receptor fusion proteins (R&D Systems) were diluted in Dulbecco's PBS (Gibco) to final concentrations of 10nM and 0.6nM, respectively. Maxisorp Nunc white 96 well microtitre plates (Nalge Nunc) were coated overnight at 4°C with 100µl/well of the diluted TrkA or p75 receptor solution. Plates were washed 3 times with PBS Tween 20, and then blocked with 200µl/well 3% w/v bovine serum albumin (BSA) in PBS. Plates were washed after 1h incubation at room temperature. Test antibodies were diluted to the desired concentration in assay buffer (0.5%w/v BSA and 0.05%v/v Tween 20 in PBS). An irrelevant antibody, not directed towards NGF, was used as a negative control whilst non-radiolabelled NGF was used as a reference inhibitor of radioligand binding. Duplicate wells were prepared for each concentration of test sample. Human or rat ^{125}I-NGF (Amersham Biosciences) was diluted with assay buffer such that the final concentration in assay wells, when mixed with test sample, was 150pM in a total assay volume of 100µl. Assay plates were incubated at room temperature for 2h before washing with PBS/Tween 20 to remove unbound ^{125}I-NGF. Bound radiolabel was quantified by addition of 100µl/well Microscint 20 (Perkin Elmer) followed by counting using a Packard TopCount microplate liquid scintillation counter. Data were plotted and analysed using Graphpad Prism software to calculate IC_{50} values for each experiment, and to derive the corresponding Ki according to the Cheng-Prusoff equation;

$$Ki = IC_{50} / (1 + D / Kd)$$

Where:

D = the NGF concentration in the assay (nominally 150pM, but actual assay concentrations were determined for each experiment)

Kd = the affinity of NGF for the TrkA or p75 receptor under identical assay conditions. This was determined by saturation binding analysis of ^{125}I-NGF binding to TrkA and p75 receptors in separate experiments.

All antibodies evaluated, except the human IgG4 control, inhibited human and rat ^{125}I-NGF binding to human TrkA and p75 receptors (Figures 8 and 9; Table 5). Table 6 shows IC50 and Ki values calculated from data shown in Figure 8A; Table 7 shows IC50 and Ki values calculated from data shown in Figure 8B; Table 8 shows IC50 and Ki values calculated from data shown in Figure 9A; Table 9 shows IC50 and Ki values calculated from data shown in Figure 9B. Antibody 1252A5 consistently showed the greatest potency of ^{125}I-NGF binding inhibition. Interestingly, binding inhibition constant values determined for 1252A5-mediated inhibition of NGF binding to TrkA and p75 receptors were significantly different. Thus, the calculated mean pKi for inhibition of human NGF binding to TrkA and p75 was 10.26 ± 0.08 and 9.85 ± 0.04, respectively ($P<0.01$, Student's T-test; both n=3). Calculated mean pKi for inhibition of rat NGF to TrkA and p75 receptors was 9.79 ± 0.04 and 9.55 ± 0.03, respectively ($P<0.05$, Student's T-test; both n=3). This result suggests that 1252A5 is a preferential inhibitor of the interaction between NGF and the TrkA receptor, and unexpectedly contrasts with the results obtained with G1152H5 for which there was no significant difference between corresponding pKi values (Table 5).

EXAMPLE 10

Antihyperalgesic activity of human IgG4 NGF antibodies

The antihyperalgesic activity of NGF antibodies was evaluated in a mouse model of carrageenan-induced thermal hypersensitivity. Male mice (20-25g body weight) were initially acclimatised to the test apparatus for 2h. The following day, baseline measures of responsiveness to thermal stimulation of both hind paws were determined. A focussed heat source was applied to the plantar hind paw surface, and the latency to withdrawal was recorded, according to the method of Hargreaves *et al.* (1988). Baseline values were calculated as the mean of triplicate determinations for each paw, recorded 10 min apart. Mice then received an intraperitoneal injection of NGF-neutralising human IgG4 antibody or control isotype-matched null antibody in phosphate-buffered saline (PBS) vehicle. Twenty-four hours later, inflammatory hyperalgesia was induced by subplantar injection of carrageenan (2% w/v in PBS; 30µl injection volume). After a further 24h period, withdrawal latencies were again determined for inflamed and non-inflamed hind paws.

Thermal hyperalgesia observed 24h after carrageenan injection was dose-dependently inhibited by pretreatment of mice with the human IgG4 NGF antibody 1252A5 (Figure 10).

All documents cited are incorporated herein by reference.

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Table 1

Neutralising potency of human IgG4 NGF antibodies in the calcium mobilisation assay

Antibody	Human NGF IC_{50} (nM)	Rat NGF IC_{50} (nM)
Mab256	0.76 ± 0.15 [a]	0.79 ± 0.08 [b]
1064F8	2.5 ± 0.6 [c]	5.5
1022E3	18 ± 6 [c]	14
1083H4	30	61 [e]
1021E5	76	75 [e]
1033G9	55 ± 20 [c]	26
1016A8	65 ± 12 [b]	14
1028F8	73	ND
1033B2	85	ND
1024C4	410 ± 120* [c]	565*
1057F11	3700*	ND

Data indicate mean of two separate determinations, except [a]n=14, [b]n=12, [c]n=3, [d]n=4 (mean ± sem) and [e]n=1. *Values determined by extrapolation. ND = not determined.

Table 2a CDRs of 1021E5-derived optimised clones containing the HCDR3 sequence LNPSLTA (SEQ ID NO: 531)

	HCDR1					HCDR2																	HCDR3																		
Kabat numbering	31	32	33	34	35	50	51	52	52A	53	54	55	56	57	58	59	60	61	62	63	64	65	95	96	97	98	99	100	100A	100B	100C	100D	100E	100F	100G	100H	100I	100J	100K	101	102
LOT1021E05	T	Y	G	I	S	G	I	I	P	I	F	D	T	G	N	S	A	Q	S	F	Q	G	S	S	R	I	Y	D	Y	A	G	G	D	H	Y	Y	Y	D	M	D	V
LOT1131H02																													L	N	P	S	L	T	A						
LOT1132H09																													L	N	P	S	L	T	A						
LOT1133C11																													L	N	P	S	L	T	A						
LOT1134D09																													L	N	P	S	L	T	A						
LOT1145D07																													L	N	P	S	L	T	A						
LOT1147A03													A																L	N	P	S	L	T	A						
LOT1147D02				V																									L	N	P	S	L	T	A						
LOT1147F02							V																	N					L	N	P	S	L	T	A						
LOT1147G09	A																												L	N	P	S	L	T	A						
LOT1149D09																													L	N	P	S	L	T	A						
LOT1150D09																													L	N	P	S	L	T	A						
LOT1150F01																						D							L	N	P	S	L	T	A						
LOT1150G08																													L	N	P	S	L	T	A						
LOT1152D05																													L	N	P	S	L	T	A						
LOT1152G10														V															L	N	P	S	L	T	A						
LOT1156G08															S														L	N	P	S	L	T	A						
LOT1160E03																													L	N	P	S	L	T	A						
LOT1165D04																													L	N	P	S	L	T	A						
LOT1215A05												N																	L	N	P	S	L	T	A						
LOT1216H05															S														L	N	P	S	L	T	A						
LOT1219D09																													L	N	P	S	L	T	A						

	linker	LCDR1													LCDR2							LCDR3											scFv IC50 in hu NGF binding assay (nM)	scFv IC50 in rat NGF binding assay (nM)	N
Kabat numbering		24	25	26	27	27A	27B	28	29	30	31	32	33	34	50	51	52	53	54	55	56	89	90	91	92	93	94	95	95A	95B	96	97			
LOT1021E05		S	G	S	S	S	N	I	G	N	N	Y	V	S	D	N	N	K	R	P	S	G	T	W	D	S	S	L	S	A	W	V	94	269	5
LOT1131H02				T																													<0.01	0.09	1
LOT1132H09							D														T												0.64	0.68	2
LOT1133C11																																	1.93	1.24	2
LOT1134D09																											G						0.14	0.20	4
LOT1145D07						T																											0.23	0.02	2
LOT1147A03				G																													2.38	0.14	1
LOT1147D02																																	<0.01	0.10	1
LOT1147F02																																	0.76	1.04	2
LOT1147G09																																	0.25	0.01	2
LOT1149D09																							A										0.01	0.12	1
LOT1150D09										D																							0.70	0.14	1
LOT1150F01																																	0.20	0.16	2
LOT1150G08				G																													0.20	0.54	1
LOT1152D05								T																									0.67	0.06	1
LOT1152G10						P																											0.23	0.05	2
LOT1156G08																																	0.32	1.05	1
LOT1160E03					N																												0.14	0.22	1
LOT1165D04									E																								0.11	0.29	1
LOT1215A05																																	1.43	0.06	1
LOT1216H05								T																									<0.01	0.03	1
LOT1219D09					R																												0.06	0.11	1

Columns on the right hand side of the table show an estimate of NGF neutralising potencies (IC50) for each clone. Purified scFv were assayed in an NGF-binding assay as described in Example 3.

Table 2b SEQ ID NOS corresponding to CDR sequences of clones shown in Table 2a

	HCDR1	HCDR2	HCDR3	LCDR1	LCDR2	LCDR3
LOT1021E05	SEQ ID NO: 33	SEQ ID NO: 34	SEQ ID NO: 35	SEQ ID NO: 38	SEQ ID NO: 39	SEQ ID NO: 40
LOT1131H02	SEQ ID NO: 163	SEQ ID NO: 164	SEQ ID NO: 165	SEQ ID NO: 168	SEQ ID NO: 169	SEQ ID NO: 170
LOT1132H09	SEQ ID NO: 183	SEQ ID NO: 184	SEQ ID NO: 185	SEQ ID NO: 188	SEQ ID NO: 189	SEQ ID NO: 190
LOT1133C11	SEQ ID NO: 193	SEQ ID NO: 194	SEQ ID NO: 195	SEQ ID NO: 198	SEQ ID NO: 199	SEQ ID NO: 200
LOT1134D09	SEQ ID NO: 203	SEQ ID NO: 204	SEQ ID NO: 205	SEQ ID NO: 208	SEQ ID NO: 209	SEQ ID NO: 210
LOT1146D07	SEQ ID NO: 223	SEQ ID NO: 224	SEQ ID NO: 225	SEQ ID NO: 228	SEQ ID NO: 229	SEQ ID NO: 230
LOT1147A03	SEQ ID NO: 433	SEQ ID NO: 434	SEQ ID NO: 435	SEQ ID NO: 438	SEQ ID NO: 439	SEQ ID NO: 440
LOT1147D02	SEQ ID NO: 233	SEQ ID NO: 234	SEQ ID NO: 235	SEQ ID NO: 238	SEQ ID NO: 239	SEQ ID NO: 240
LOT1147F02	SEQ ID NO: 443	SEQ ID NO: 444	SEQ ID NO: 445	SEQ ID NO: 448	SEQ ID NO: 449	SEQ ID NO: 450
LOT1147G09	SEQ ID NO: 243	SEQ ID NO: 244	SEQ ID NO: 245	SEQ ID NO: 248	SEQ ID NO: 249	SEQ ID NO: 250
LOT1149D09	SEQ ID NO: 453	SEQ ID NO: 454	SEQ ID NO: 455	SEQ ID NO: 458	SEQ ID NO: 459	SEQ ID NO: 460
LOT1150D09	SEQ ID NO: 463	SEQ ID NO: 464	SEQ ID NO: 465	SEQ ID NO: 468	SEQ ID NO: 469	SEQ ID NO: 470
LOT1150F01	SEQ ID NO: 253	SEQ ID NO: 254	SEQ ID NO: 255	SEQ ID NO: 258	SEQ ID NO: 259	SEQ ID NO: 260
LOT1150G08	SEQ ID NO: 473	SEQ ID NO: 474	SEQ ID NO: 475	SEQ ID NO: 478	SEQ ID NO: 479	SEQ ID NO: 480
LOT1152D05	SEQ ID NO: 483	SEQ ID NO: 484	SEQ ID NO: 485	SEQ ID NO: 488	SEQ ID NO: 489	SEQ ID NO: 490
LOT1152G10	SEQ ID NO: 493	SEQ ID NO: 494	SEQ ID NO: 495	SEQ ID NO: 498	SEQ ID NO: 499	SEQ ID NO: 500
LOT1156G06	SEQ ID NO: 503	SEQ ID NO: 504	SEQ ID NO: 505	SEQ ID NO: 508	SEQ ID NO: 509	SEQ ID NO: 510
LOT1160E03	SEQ ID NO: 293	SEQ ID NO: 294	SEQ ID NO: 295	SEQ ID NO: 298	SEQ ID NO: 299	SEQ ID NO: 300
LOT1165D04	SEQ ID NO: 303	SEQ ID NO: 304	SEQ ID NO: 305	SEQ ID NO: 308	SEQ ID NO: 309	SEQ ID NO: 310
LOT1215A06	SEQ ID NO: 513	SEQ ID NO: 514	SEQ ID NO: 515	SEQ ID NO: 518	SEQ ID NO: 519	SEQ ID NO: 520
LOT1218H05	SEQ ID NO: 353	SEQ ID NO: 354	SEQ ID NO: 355	SEQ ID NO: 358	SEQ ID NO: 359	SEQ ID NO: 360
LOT1219D09	SEQ ID NO: 523	SEQ ID NO: 524	SEQ ID NO: 525	SEQ ID NO: 528	SEQ ID NO: 529	SEQ ID NO: 530

Table 3
NGF-neutralising potencies of optimised human IgG4 antibodies in three assays of NGF function in whole cells

IgG		FLIPR calcium mobilisation IC_{50} (nM)			PC12 cell survival IC_{50} (nM)		TF-1 cell proliferation IC_{50} (nM)		
Clone	Parent	Human NGF	Rat NGF	Mouse NGF	Human NGF	Rat NGF	Human NGF	Rat NGF	Mouse NGF
1021E5	-	76 [a]	75	47	1300[j]				
1083H4	-	30[a]	61	54	1100[j]				
1126F1	1021E5	0.35	0.15	0.22	2.9				
1126G5	1021E5	0.23	0.16	0.16	1.50				
1126H5	1021E5	0.38	0.15	0.23	8.7				
1127D9	1021E5	0.40	0.22	0.21	0.57				
1127F9	1021E5	0.36	0.14	0.20	0.59				
1131D7	1021E5	117	37	71	670				
1131H2	1021E5	0.27	0.11	0.12	0.58				
1132A9	1021E5	0.39	0.25	0.33	0.90				
1132H9	1021E5	0.35	0.13	0.16	0.55				
1133C11	1021E5	0.45[a]	0.30[a]	0.28[a]	0.53 ± 0.13[b]	0.42 ± 0.07[b]	0.10 ± 0.01[d]	0.12 ± 0.02[d]	0.10 ± 0.02[d]
1134D9	1021E5	0.31[a]	0.14[a]	0.16[a]	0.54				
1145D1	1021E5	0.36	0.17	0.24	0.66				
1146D7	1021E5	0.38	0.33	0.31	0.48				
1147D2	1021E5	0.36	0.21	0.24	0.51				
1147G9	1021E5	0.30 ± 0.04[b]	0.21 ± 0.02[b]	0.23 ± 0.02[b]	0.76 ± 0.06[b]	0.55 ± 0.02[b]			
1150F1	1021E5	0.32	0.15	0.19	0.47				

Table 3 (cont)

IgG		FLIPR calcium mobilisation IC_{50} (nM)			PC12 cell survival IC_{50} (nM)		TF-1 cell proliferation IC_{50} (nM)		
Clone	Parent	Human NGF	Rat NGF	Mouse NGF	Human NGF	Rat NGF	Human NGF	Rat NGF	Mouse NGF
1152H5	1021E5	0.22 ± 0.05^{b}	0.21 ± 0.05^{b}	0.14 ± 0.01^{b}	0.40 ± 0.04^{e}	0.38 ± 0.04^{e}	0.26 ± 0.25^{d}	0.08 ± 0.0^{d}	0.07 ± 0.0^{d}
1155H1	1021E5	0.35	0.18	0.18	0.59				
G1152H5	1152H5	0.22	0.27	0.18	1.1 ± 0.1^{b}	7.3 ± 0.9^{b}	0.44 ± 0.17^{d}	1.44 ± 0.27^{d}	0.99 ± 0.29^{d}
1158A1	1021E5	0.34	0.12	0.11	0.48				
1160E3	1021E5	0.33	0.13	0.12	0.40				
1165D4	1021E5	0.26 ± 0.02^{b}	0.15 ± 0.01^{b}	0.16 ± 0.04^{b}	0.41 ± 0.08^{e}	0.41 ± 0.06^{e}			
G1165D4	1165D4	0.32^{a}	0.33^{a}	0.27^{a}	0.86 ± 0.04^{b}	2.63 ± 0.03^{b}			
1175H8	1021E5	0.37	0.15	0.16	1.1				
1211G10	1021E5	0.37	0.16	0.15	0.58				
1214A1	1021E5	0.26^{a}	0.16^{a}	0.14^{a}	0.52 ± 0.07^{b}	0.43 ± 0.07^{b}			
1214D10	1021E5	0.29	0.14	0.13	0.35				
1218H5	1021E5	0.33	0.13	0.15	0.47				
1227H8	1083H4	0.44^{a}	0.43^{a}	0.48^{a}	0.70 ± 0.15^{b}	35 ± 10^{b}			
1230D8	1083H4	0.31^{a}	0.29^{a}	0.37^{a}	0.71 ± 0.14^{b}	37 ± 4^{b}			
1230H7	1021E5	0.27 ± 0.04^{b}	0.18 ± 0.03^{b}	0.15 ± 0.02^{b}	0.42 ± 0.10^{c}	0.32 ± 0.05^{c}			
G1230H7	1230H7	0.31^{a}	0.34^{a}	0.25^{a}	2.5 ± 0.2^{b}	7.5 ± 0.4^{b}			
1252A5	1133C11	0.33 ± 0.03^{c}	0.29 ± 0.06^{c}	0.26 ± 0.01^{c}	0.94 ± 0.13^{f}	2.7 ± 0.6^{f}	$0.42 \pm .15^{d}$	0.72 ± 0.40^{d}	0.55 ± 0.32^{d}
Mab 256	-	0.76 ± 0.15^{h}	0.79 ± 0.08^{g}	1.4 ± 0.2^{i}	23 ± 1^{j}	44 ± 7^{f}	6 ± 4^{d}	5 ± 3^{d}	7 ± 3^{d}

Data are n=1 except; [a]n=2; [b]n=3; [c]n=4; [d]n=5; [e]n=6; [f]n=7; [g]n=12; [h]n=14; [i]n=15; [j]extrapolated

Table 4
Cross-reactivity of optimised human IgG4 NGF antibodies with other neurotrophins

IgG	BDNF, %	NT-3, %	NT-4, %
1133C11	0.7-3.1	0.9-1.8	0-1.7
1147G9	0-1.3	0.1-0.9	0-1.3
1152H5	0-1.5	0-0.5	0.2-1.2
1165D4	0-1.3	0-0.7	0-1.5
1214A1	0-1.4	0-1.0	0-1.0
1230H7	0-1.1	0-0.8	0-0.7
1252A5	0-1.4	0-1.1	0-0.9

Data in columns show the range of calculated antibody cross-reactivities. Values are calculated as percentage of the signal observed against each neurotrophin, relative to the NGF binding signal at the same test antibody concentration. Neurotrophins were coated to assay plates at a concentration of 100ng/well, and binding of test antibodies was measured over the concentration range 0.03 - 10nM. Data represent the result of a single experiment.

Table 5
Summary of binding inhibition constant determinations for 1252A5 and G1152H5. Data represent mean ± s.e.m. of three independent experiments.

IgG	pKi vs human NGF		pKi vs rat NGF	
	TrkA	p75	TrkA	p75
1252A5	10.26 ± 0.08*	9.85 ± 0.04	9.79 ± 0.04**	9.55 ± 0.03
G1152H5	9.59 ± 0.08§	9.56 ± 0.04	9.18 ± 0.08§	9.24 ± 0.05

* P<0.01 c.f. hu NGF / p75 interaction
** P<0.05 c.f. rat NGF / p75 interaction
§ N/S c.f. p75
Student's unpaired T-test

Table 6

IgG	IC_{50} (nM)	Ki (nM)
NGF	2.4	2.1
1252A5	0.068	0.061
G1152H5	0.204	0.184
MAB256	1.94	1.76
MAB5260Z	0.368	0.333

Table 7

IgG	IC_{50} (nM)	Ki (nM)
NGF	1.3	1.2
1252A5	0.151	0.140
G1152H5	0.538	0.499
MAB256	1.48	1.38
MAB5260Z	0.310	0.288

Table 8

IgG	IC_{50} (nM)	Ki (nM)
NGF	0.686	0.568
1252A5	0.188	0.155
G1152H5	0.348	0.288
MAB256	0.304	0.252
MAB5260Z	0.570	0.472

Table 9

IgG	IC_{50} (nM)	Ki (nM)
NGF	0.783	0.710
1252A5	0.302	0.274
G1152H5	0.781	0.708
MAB256	2.94	2.67
MAB5260Z	0.587	0.532

CLAIMS:

1. An isolated specific binding member for nerve growth factor (NGF), comprising an antibody antigen-binding site which is composed of a human antibody VH domain and a human antibody VL domain and which comprises a set of CDRs HCDR1, HCDR2, HCDR3, LCDR1, LCDR2 and LCDR3, wherein the VH domain comprises HCDR 1, HCDR2, HCDR3 and a framework and the VL domain comprises LCDR1, LCDR2, LCDR3 and a framework, wherein the set of CDRs consists of a set of CDRs selected from the group consisting of:

the 1133C11 set of CDRs, defined wherein the HCDR1 has the amino acid sequence of SEQ ID NO: 193, the HCDR2 has the amino acid sequence of SEQ ID NO: 194, the HCDR3 has the amino acid sequence of SEQ ID NO: 195, the LCDR1 has the amino acid sequence of SEQ ID NO: 198, the LCDR2 has the amino acid sequence of SEQ ID NO: 199, and the LCDR3 has the amino acid sequence of SEQ ID NO: 200;

a set of CDRs which contains one or two amino acid substitutions compared with the 1133C11 set of CDRs;

each set of CDRs as shown for individual clones in Table 2; and

a set of CDRs which contains the 113C11 set of CDRs with the amino acid sequence MISSLQP (SEQ ID NO: 533) or the amino acid sequence FNSALIS (SEQ ID NO: 532) substituted for the amino acid sequence LNPSLTA (SEQ ID NO: 531) within HCDR3.

2. An isolated specific binding member according to claim 1 wherein the one or two substitutions are at one or two of the following residues within the CDRs, using the standard numbering of Kabat:

31, 34 in HCDR1

51, 55, 56, 57, 58, 65 in HCDR2

96 in HCDR3

26, 27, 27A, 27B, 28, 29, 30 in LCDR1

56 in LCDR2

90, 94 in LCDR3.

3. An isolated specific binding member according to claim 2 wherein the one or two substitutions are made at the following positions from among the identified groups of possible substitute residues for each position:

Position of substitution	Substitute Residue selected from the group consisting of
31 in HCDR1:	A
34 in HCDR1:	V
51 in HCDR2:	V
55 in HCDR2:	N
56 in HCDR2:	A
57 in HCDR2:	V
58 in HCDR2:	S
65 in HCDR2:	D
96 in HCDR3:	N
26 in LCDR1:	T
26 in LCDR1:	G
27 in LCDR1:	N
27 in LCDR1:	R
27A in LCDR1:	T
27A in LCDR1:	P
27B in LCDR1:	D

28 in LCDR1: T
29 in LCDR1: E
30 in LCDR1: D

56 in LCDR2: T

90 in LCDR3: A
94 in LCDR3: G.

4. An isolated specific binding member according to claim 3 wherein residue 29 within LCDR1 is E.

5. An isolated specific binding member according to claim 1 comprising a set of CDRs which contains the 113C11 set of CDRs with the amino acid sequence MISSLQP (SEQ ID NO: 533) substituted for the amino acid sequence LNPSLTA (SEQ ID NO: 531) within HCDR3.

6. An isolated specific binding member according to claim 1 comprising a set of CDRs which contains the 113C11 set of CDRs with the amino acid sequence FNSALIS (SEQ ID NO: 532) substituted for the amino acid sequence LNPSLTA (SEQ ID NO: 531) within HCDR3.

7. An isolated specific binding member according to claim 1 comprising the 1133C11 set of CDRs.

8. An isolated specific binding member according to any one of claims 1 to 7 wherein the VH domain framework is human heavy chain germline framework and/or the VL domain framework is human light chain germline framework.

9. An isolated specific binding member according to claim 8 wherein the heavy chain germline framework comprises VH1 DP10.

10. An isolated specific binding member according to claim 8 or claim 9 wherein the light chain germline framework comprises VL Vλ1.

11. An isolated specific binding member according to any one of claims 1 to 10 which binds rat or mouse NGF.

12. An isolated specific binding member according to any one of claims 1 to 11 which preferentially blocks NGF binding to TrkA receptor over NGF binding to p75 receptor.

13. A specific binding member according to any one of claims 9 to 12 comprising the 1252A5 VH domain (SEQ ID NO: 392).

14. A specific binding member according to any one of claims 9 to 13 comprising the 1252A5 VL domain (SEQ ID NO: 397).

15. A specific binding member according to any one of claims 1 to 14 that binds human NGF with affinity equal to or better than the affinity of an antigen-binding site for human NGF formed by the 1252A5 VH domain (SEQ ID NO: 392) and the 1252A5 VL domain (SEQ ID NO: 397), the affinity of the specific binding member and the affinity of the antigen-binding site being as determined under the same conditions.

16. A specific binding member according to any one of claims 1 to 15 that binds to and/or neutralises human NGF.

17. A specific binding member according to claim 16 that neutralizes human NGF, with a potency equal to or better than the potency of a NGF antigen-binding site formed by the 1252A5 VH domain (SEQ ID NO: 392) and the 1252A5 VL domain (SEQ ID NO: 397), the potency of the specific binding member and the potency of the antigen-binding site being as determined under the same conditions.

18. A specific binding member according to any one of claims 9 to 11 comprising the 1152H5 VH domain (SEQ ID NO: 262).

19. A specific binding member according to any one of claims 9 to 11 or claim 18 comprising the 1152H5 VL domain (SEQ ID NO: 267).

20. A specific binding member according to any one of claims 9 to 11 comprising the 1165D4 VH domain (SEQ ID NO: 302).

21. A specific binding member according to any one of claims 9 to 11 or claim 20 comprising the 1165D4 VL domain (SEQ ID NO: 307).

22. A specific binding member according to any one of claims 9 to 11 comprising the 1230H7 VH domain (SEQ ID NO: 362).

23. A specific binding member according to any one of claims 9 to 11 or claim 22 comprising the 1230H7 VL domain (SEQ ID NO: 367).

24. A specific binding member according to any one of claims 1 to 23 that comprises an scFv antibody molecule.

25. A specific binding member according to any one of claims 1 to 23 that comprises an antibody constant region.

26. A specific binding member according to claim 25 that comprises a whole antibody.

27. A specific binding member according to claim 26 wherein the whole antibody is IgG4.

28. An isolated antibody VH domain of a specific binding member according to any one of claims 1 to 23.

29. An isolated antibody VL domain of a specific binding member according to any one of claims 1 to 23.

30. A composition comprising a specific binding member, antibody VH domain or antibody VL according to any one of claims 1 to 29 and at least one additional component.

31. A composition according to claim 30 comprising a pharmaceutically acceptable excipient, vehicle or carrier.

32. An isolated nucleic acid which comprises a nucleotide sequence encoding a specific binding member or antibody VH or VL domain of a specific binding member according to any one of claims 1 to 29.

33. A host cell *in vitro* transformed with nucleic acid according to claim 32.

34. A method of producing a specific binding member or antibody VH or VL domain, the method comprising culturing host cells according to claim 33 under conditions for production of said specific binding member or antibody VH or VL domain.

35. A method according to claim 34 further comprising isolating and/or purifying said specific binding member or antibody VH or VL variable domain.

36. A method according to claim 34 or claim 35 further comprising formulating the specific binding member or antibody VH or VL variable domain into a composition including at least one additional component.

37. A method for producing an antibody antigen-binding domain for NGF, the method comprising

providing, by way of addition, deletion, substitution or insertion of one or more amino acids in the amino acid sequence of a parent VH domain comprising HCDR 1, HCDR2 and HCDR3, wherein the parent VH domain HCDR1, HCDR2 and HCDR3 are the 1252A5 set of HCDRs, defined wherein the HCDR1 has the amino acid sequence of SEQ ID NO: 393, the HCDR2 has the amino acid sequence of SEQ ID NO: 394, the HCDR3 has the amino acid sequence of SEQ ID NO: 395, or the 1021E5 set of HCDRs, defined wherein the HCDR1 has the amino acid sequence of SEQ ID NO: 33, the HCDR2 has the amino acid sequence of SEQ ID NO: 34, the HCDR3 has the amino acid sequence of SEQ ID NO: 35, a VH domain which is an amino acid sequence variant of the parent VH domain, and optionally combining the VH domain thus provided with one or more VL domains to provide one or more VH/VL combinations; and

testing said VH domain which is an amino acid sequence variant of the parent VH domain or the VH/VL combination or combinations to identify an antibody antigen binding domain specific for NGF.

38. A method according to claim 37 wherein the parent VH domain amino acid sequence is selected from the group consisting of SEQ ID NO: 392 and SEQ ID NO: 32.

39. A method according to claim 37 or claim 38 wherein said one or more VL domains is provided by way of addition, deletion, substitution or insertion of one or more amino acids in the amino acid sequence of a parent VL domain comprising LCDR 1, LCDR2 and LCDR3, wherein the parent VL domain LCDR1, LCDR2 and LCDR3 are the 1252A5 set of LCDRs, defined wherein the LCDR1 has the amino acid sequence of SEQ ID NO: 398, the LCDR2 has the amino acid sequence of SEQ ID NO: 399, the LCDR3 has the amino acid sequence of SEQ ID NO: 400, or the 1021E5 set of LCDRs, defined wherein the LCDR1 has the amino acid sequence of SEQ ID NO: 38, the LCDR2 has the amino acid sequence of SEQ ID NO: 39, the LCDR3 has the amino acid sequence of SEQ ID NO: 40, producing one or more VL domains each of which is an amino acid sequence variant of the parent VL domain.

40. A method according to claim 39 wherein the parent VL domain amino acid sequence is selected from the group consisting of SEQ ID NO: 397 and SEQ ID NO: 37.

41. A method according to any of claims 37 to 40, wherein the NGF is human NGF.

42. A method for producing an antibody antigen-binding domain for NGF, the method comprising

providing, by way of addition, deletion, substitution or insertion of one or more amino acids in the amino acid sequence of a parent VH domain comprising HCDR 1, HCDR2 and HCDR3, wherein the parent VH domain HCDR1, HCDR2 and HCDR3 are the 1152H5 set of HCDRs, defined wherein the HCDR1 has the amino acid sequence of SEQ ID NO: 263, the HCDR2 has the amino acid sequence of SEQ ID NO: 264, the HCDR3 has the amino acid sequence of SEQ ID NO: 265, the 1165D4 set of HCDRs, defined wherein the HCDR1 has the amino acid sequence of SEQ ID NO: 303, the HCDR2 has the amino acid sequence of SEQ ID NO: 304, the HCDR3 has the amino acid sequence of SEQ ID NO: 305, or the 1230H7 set of HCDRs, defined wherein the HCDR1 has the amino acid sequence of SEQ ID NO: 363, the HCDR2 has the amino acid sequence of SEQ ID NO: 364, the HCDR3 has the amino acid sequence of SEQ ID NO: 365, a VH domain which is an amino acid sequence variant of the parent VH domain, and optionally combining the VH domain thus provided with one or more VL domains to provide one or more VH/VL combinations; and

testing said VH domain which is an amino acid sequence variant of the parent VH domain or the VH/VL combination or combinations to identify an antibody antigen binding domain specific for NGF.

43. A method according to claim 42 wherein the parent VH domain amino acid sequence is selected from the group consisting of SEQ ID NO: 262, SEQ ID NO: 302 and SEQ ID NO: 362.

44. A method according to claim 42 or claim 43 wherein said one or more VL domains is provided by way of addition, deletion, substitution or insertion of one or more amino acids in the amino acid sequence of a parent VL domain comprising LCDR 1, LCDR2 and LCDR3, wherein the parent VL domain LCDR1, LCDR2 and LCDR3 are the 1152H5 set of LCDRs, defined wherein the LCDR1 has the amino acid sequence of SEQ ID NO: 268, the LCDR2 has the amino acid sequence of SEQ ID NO: 269, the LCDR3 has the amino acid sequence of SEQ ID NO: 270, the 1165D4 set of LCDRs, defined wherein the LCDR1 has the amino acid sequence of SEQ ID NO: 308, the LCDR2 has the amino acid sequence of SEQ ID NO: 309, the LCDR3 has the amino acid sequence of SEQ ID NO: 310, or the 1230H7 set of LCDRs, defined wherein the LCDR1 has the amino acid sequence of SEQ ID NO: 368, the LCDR2 has the amino acid sequence of SEQ ID NO: 369, the LCDR3 has the amino acid sequence of SEQ ID NO: 370, producing one or more VL domains each of which is an amino acid sequence variant of the parent VL domain.

45. A method according to claim 44 wherein the parent VL domain amino acid sequence is selected from the group consisting of SEQ ID NO: 267, SEQ ID NO: 307 and SEQ ID NO: 367.

46. A method according to any of claims 42 to 45, wherein the NGF is human NGF.

47. A method according to any one of claims 37 to 46 wherein said VH domain which is an amino acid sequence variant of the parent VH domain is provided by CDR mutagenesis.

48. A method according to any one of claims 37 to 47 further comprising producing the antibody antigen-binding site as a component of an IgG, scFv or Fab antibody molecule.

49. A method for producing a specific binding member that binds NGF, which method comprises:

providing starting nucleic acid encoding a VH domain or a starting repertoire of nucleic acids each encoding a VH domain, wherein the VH domain or VH domains either comprise a HCDR1, HCDR2 and/or HCDR3 to be replaced or lack a HCDR1, HCDR2 and/or HCDR3 encoding region;

combining said starting nucleic acid or starting repertoire with donor nucleic acid or donor nucleic acids encoding or produced by mutation of the amino acid sequence of HCDR1 SEQ ID NO: 193, HCDR2 SEQ ID NO: 194, and/or HCDR3 SEQ ID NO: 395, 265, 305, 365 or 35 such that said donor nucleic acid is or donor nucleic acids are inserted into the CDR1, CDR2 and/or CDR3 region in the starting nucleic acid or starting repertoire, so as to provide a product repertoire of nucleic acids encoding VH domains;

expressing the nucleic acids of said product repertoire to produce product VH domains;

optionally combining said product VH domains with one or more VL domains;

selecting a specific binding member for NGF, which specific binding member comprises a product VH domain and optionally a VL domain; and

recovering said specific binding member or nucleic acid encoding it.

50. A method according to claim 49 wherein the donor nucleic acids are produced by mutation of said HCDR1 and/or HCDR2.

51. A method according to claim 49 wherein the donor nucleic acid is produced by mutation of HCDR3.

52. A method according to claim 51 comprising providing the donor nucleic acid by mutation of nucleic acid encoding the amino acid sequence of 1252A5 HCDR3 (SEQ ID NO: 395) or 1021E5 HCDR3 (SEQ ID NO: 35).

53. A method according to claim 49 comprising providing the donor nucleic acid by random mutation of nucleic acid.

54. A method according to any one of claims 49 to 53 further comprising attaching a product VH domain that is comprised within the recovered specific binding member to an antibody constant region.

55. A method according to any one of claims 49 to 53 comprising providing an IgG, scFv or Fab antibody molecule comprising the product VH domain and a VL domain.

56. A method according to any of claims 49 to 55, wherein the NGF is human NGF.

57. A method according to any one of claims 37 to 56, further comprising testing the antibody antigen-binding domain or specific binding member that binds NGF for ability to neutralize NGF.

58. A method according to claim 57 wherein a specific binding member that comprises an antibody fragment that binds and neutralizes NGF is obtained.

59. A method according to claim 58 wherein the antibody fragment is an scFv antibody molecule.

60. A method according to claim 58 wherein the antibody fragment is an Fab antibody molecule.

61. A method according to claim 59 or claim 60 further comprising providing the VH domain and/or the VL domain of the antibody fragment in a whole antibody.

62. A method according to any one of claims 37 to 61 further comprising formulating the specific binding member that binds NGF, antibody antigen-binding site or an antibody VH or VL variable domain of the specific binding member or antibody antigen-binding site that binds NGF, into a composition including at least one additional component.

63. A method according to any one of claims 37 to 62 further comprising binding a specific binding member that binds NGF to NGF or a fragment of NGF.

64. A method comprising binding a specific binding member that binds NGF according to any one of claims 1 to 27 to human NGF or a fragment of human NGF.

65. A method according to claim 63 or claim 64 wherein said binding takes place *in vitro*.

66. A method according to any of claims 63 to 65, comprising binding the specific binding member to human NGF or a fragment of human NGF.

67. A method according to any one of claims 63 to 66 comprising determining the amount of binding of specific binding member to NGF or a fragment of NGF.

68. A method according to any one of claims 37 to 67 further comprising use of the specific binding member in the manufacture of a medicament for treatment of a disease or disorder in which NGF plays a role.

69. Use of a specific binding member according to any one of claims 1 to 27 in the manufacture of a medicament for treatment of a disease or disorder in which NGF plays a role.

70. A method of treatment of a disease or disorder in which NGF plays a role, the method comprising administering a specific binding member according to any one of claims 1 to 27 to a patient with the disease or disorder or at risk of developing the disease or disorder.

71. A method or use according to claim 68, claim 69 or claim 70, wherein the disease or disorder is selected from the group consisting of pain, asthma, chronic obstructive pulmonary disease, pulmonary fibrosis, other diseases of airway inflammation, diabetic neuropathy, cardiac arrhythmias, HIV, arthritis, psoriasis and cancer.

72. A method or use according to claim 71 wherein said treatment is of pain.

Figure 1A

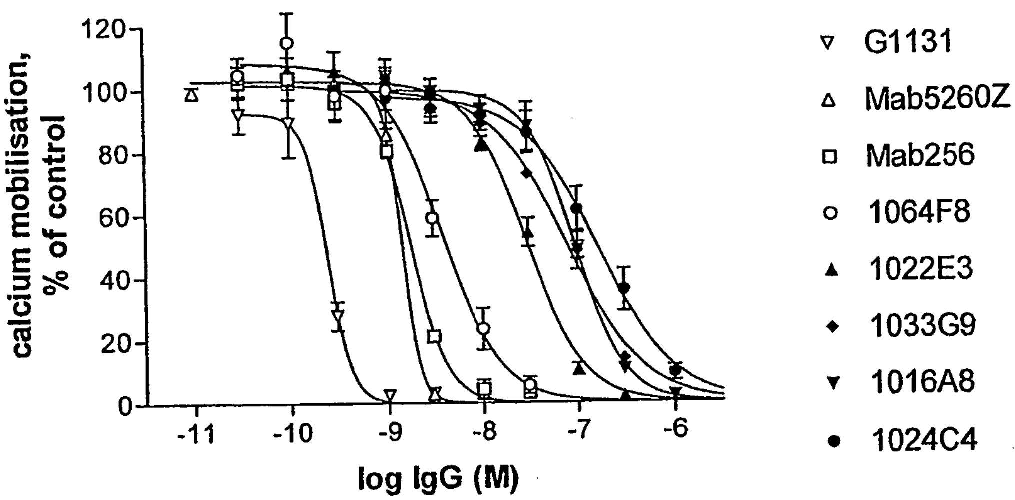


Figure 1B

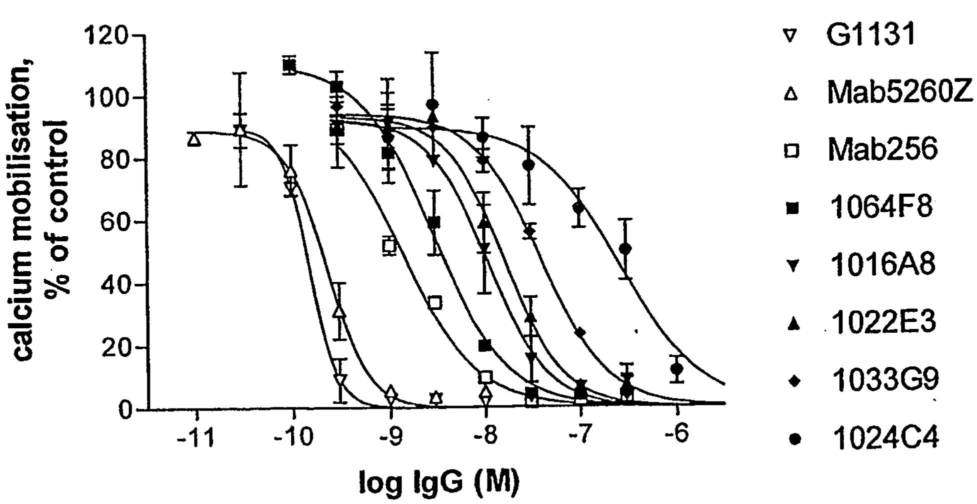


Figure 2A

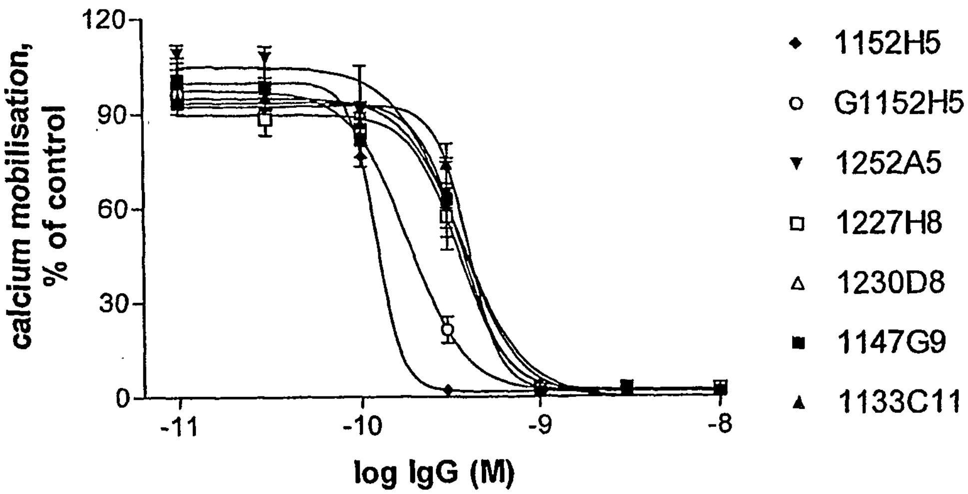


Figure 2B

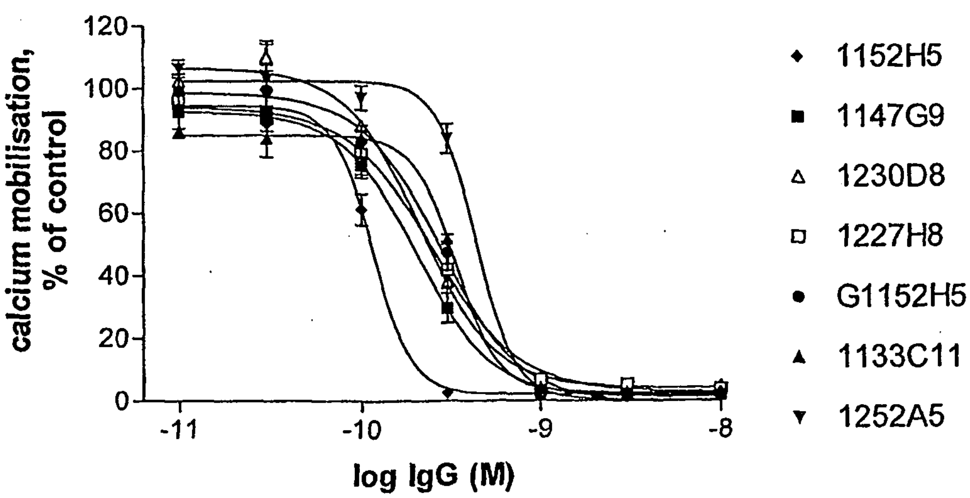


Figure 2C

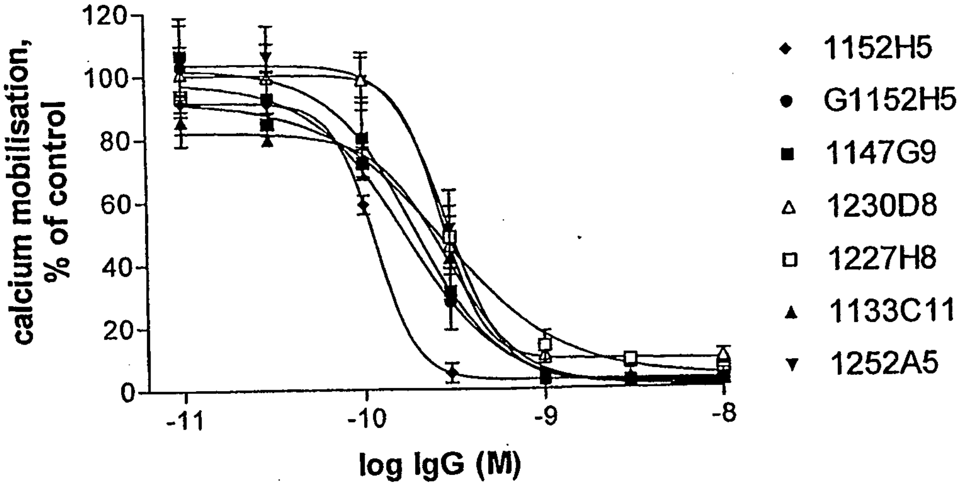

120
100
80
60
40
20
0
calcium mobilisation, % of control
-11
-10
-9
-8
log IgG (M)
1152H5
G1152H5
1147G9
1230D8
1227H8
1133C11
1252A5

Figure 3A

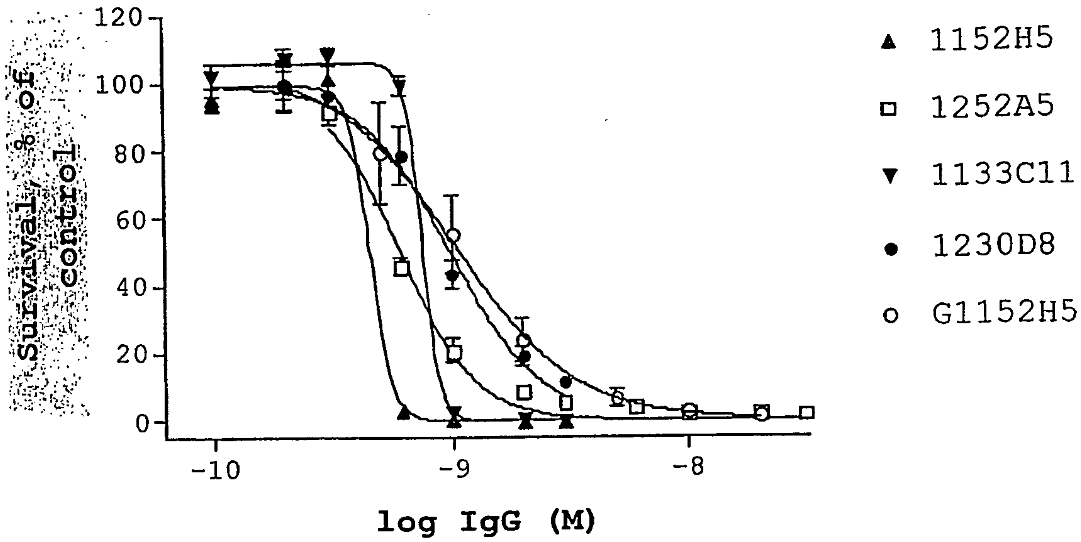


Figure 3B

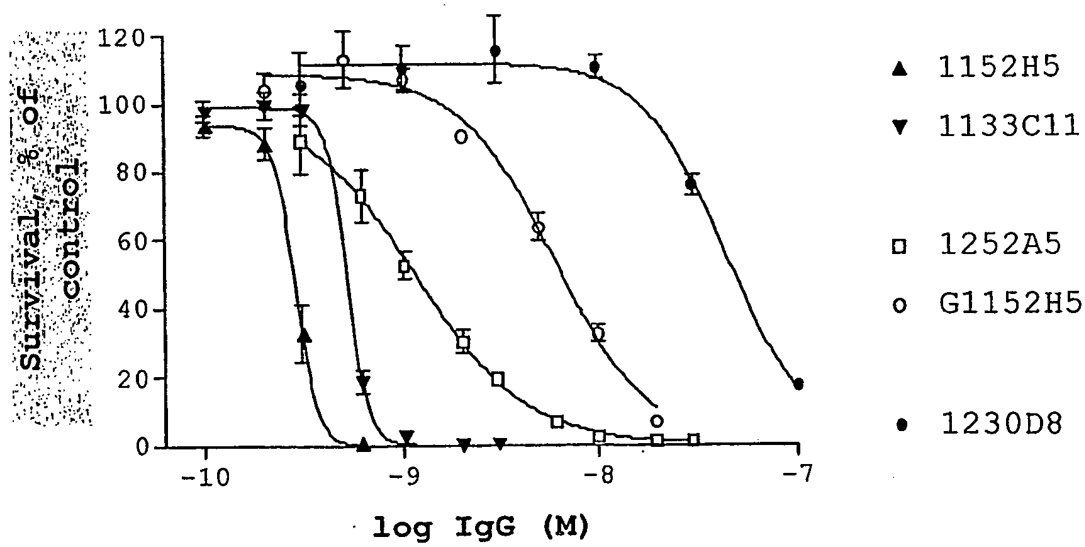


Figure 4A

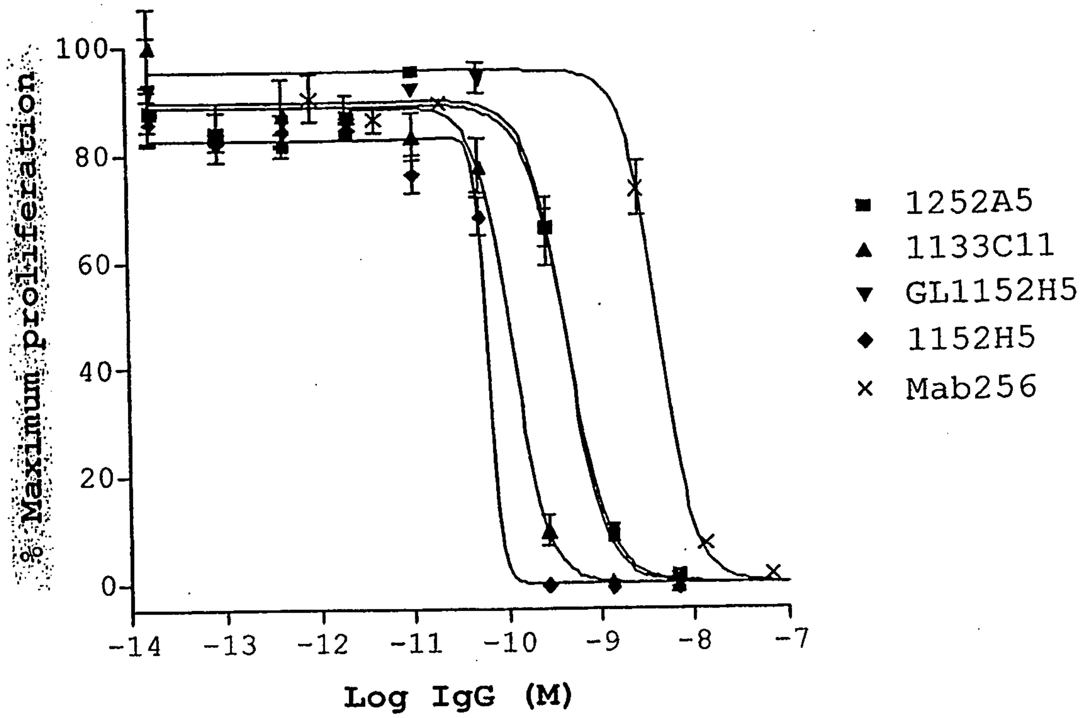


Figure 4B

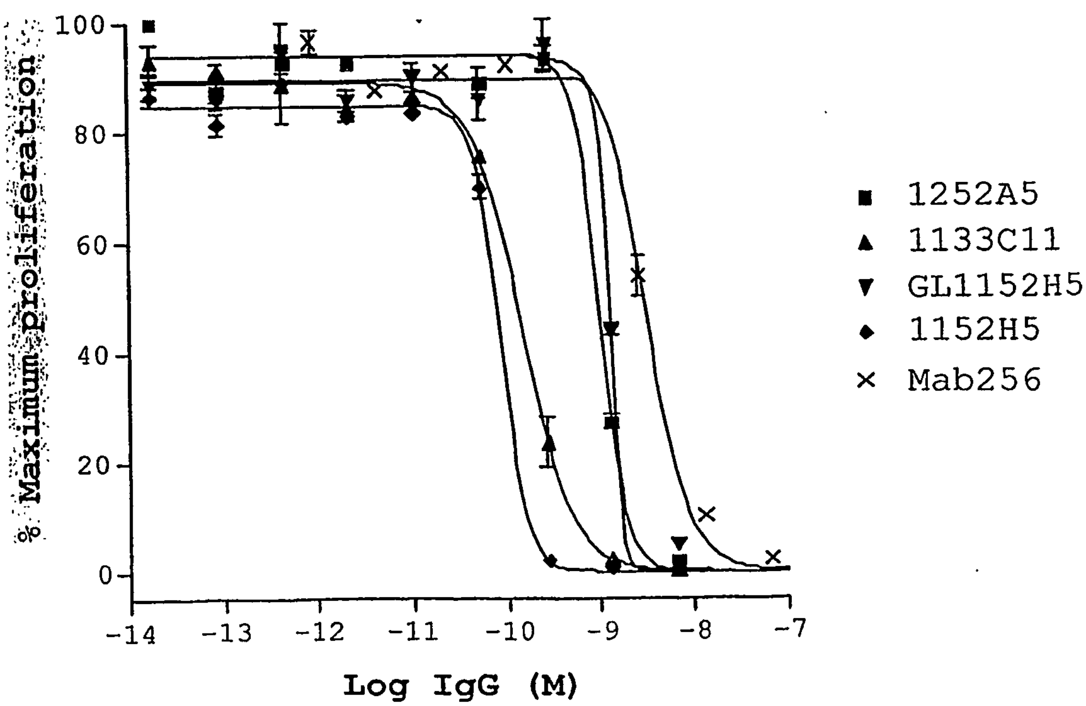
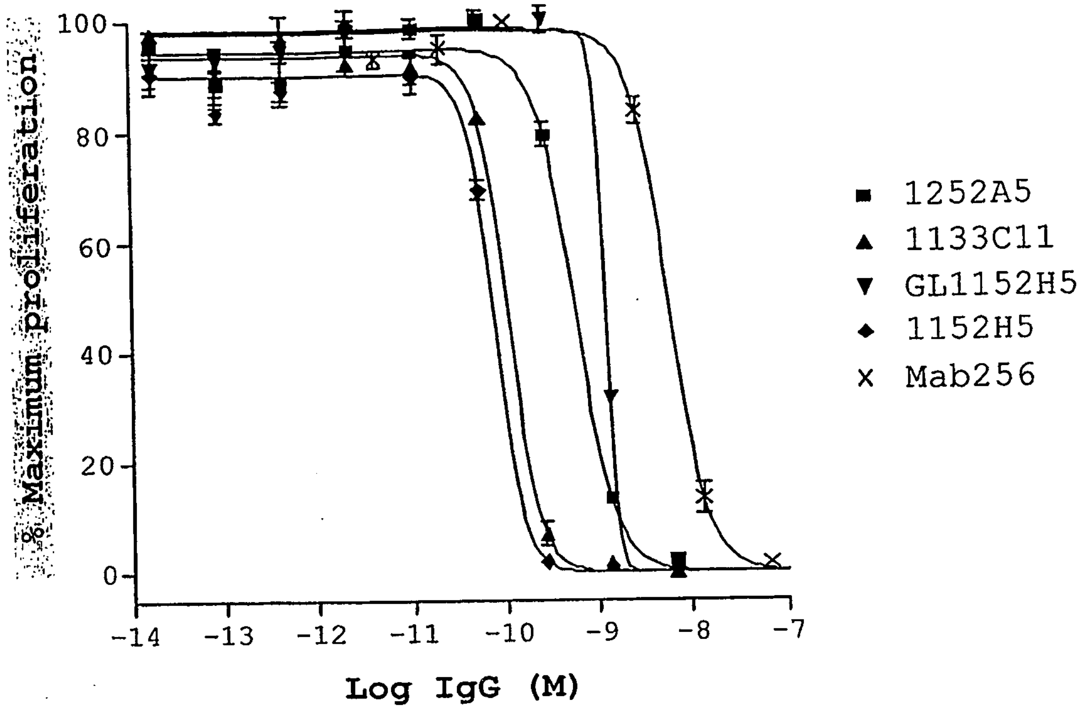


Figure 4C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20
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−14
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−11
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−7
Log IgG (M)
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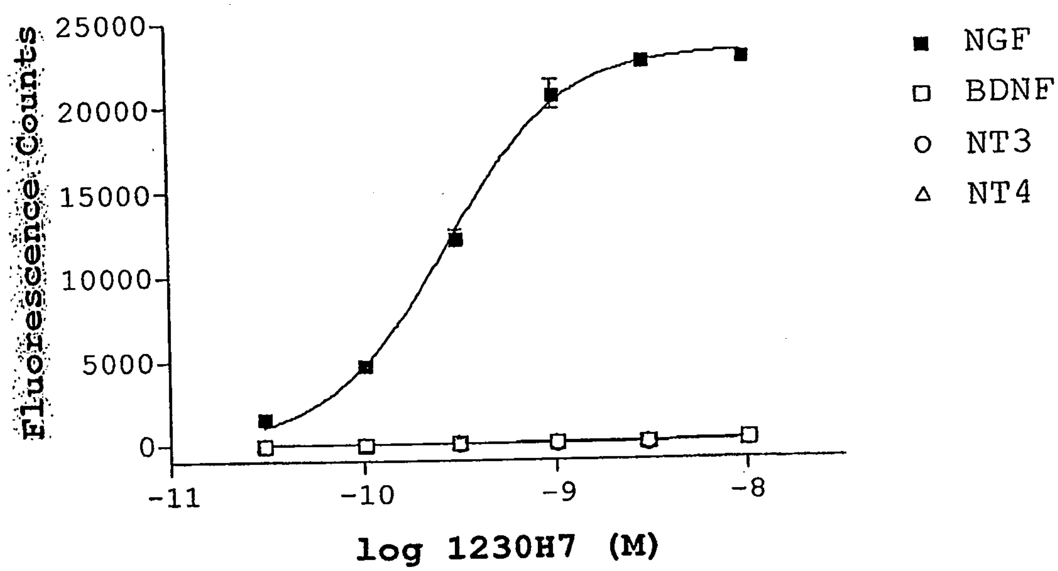
1133C11
GL1152H5
1152H5
Mab256

FIGURE 5


Fluorescence Counts
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20000
15000
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-9
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log 1230H7 (M)
NGF
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NT3
NT4

Figure 6A

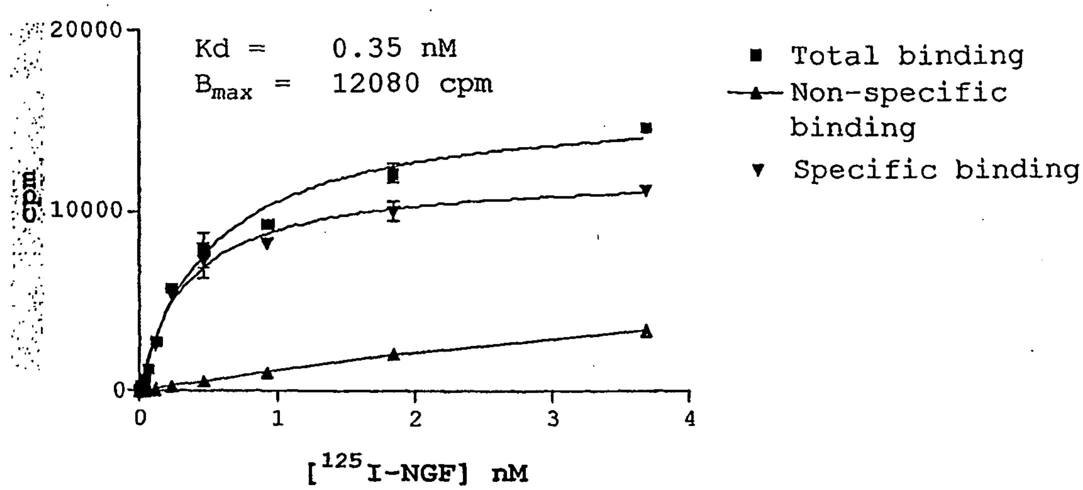


Figure 6B

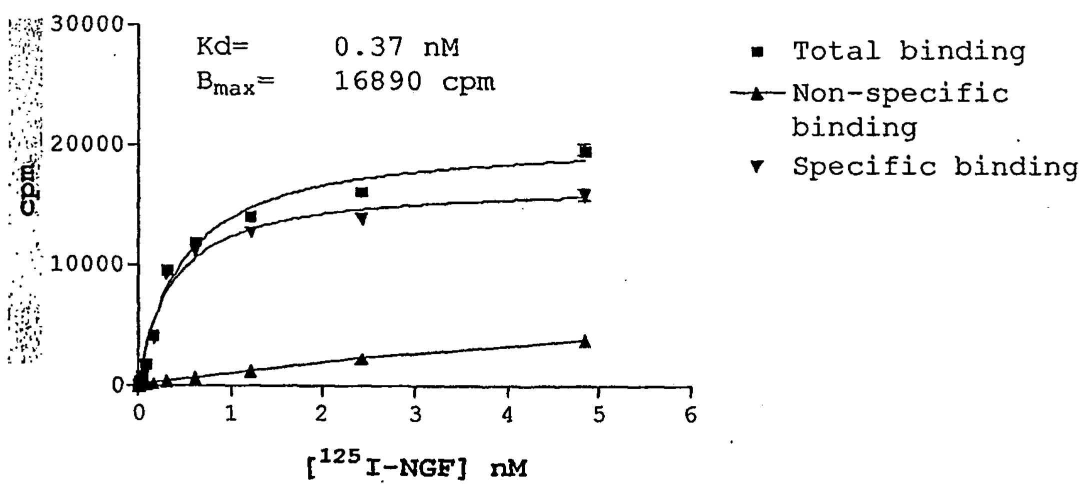


Figure 7A

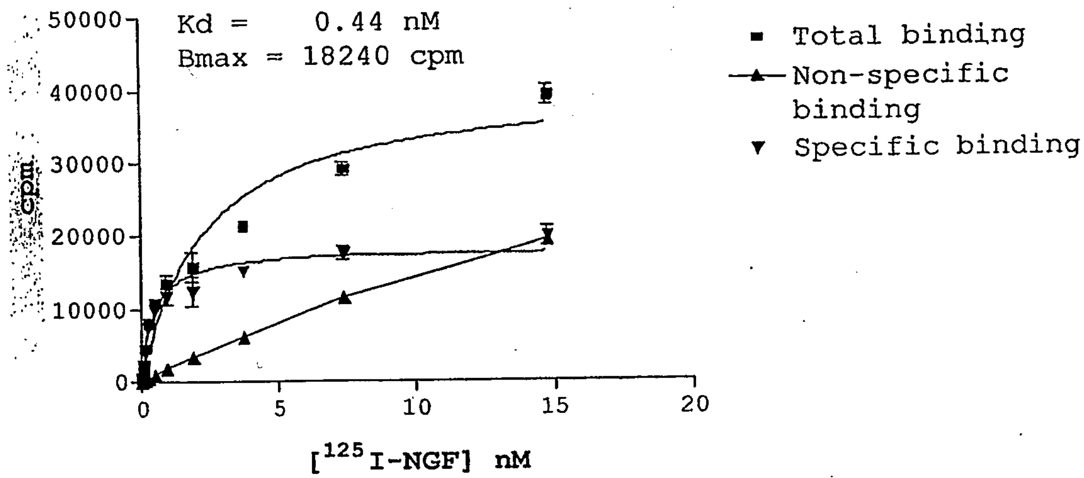


Figure 7B

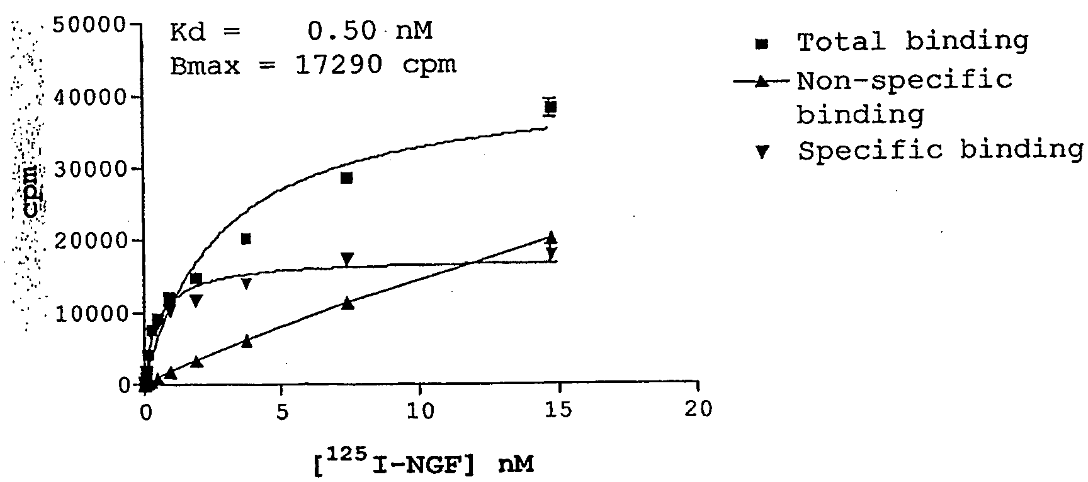


Figure 8A

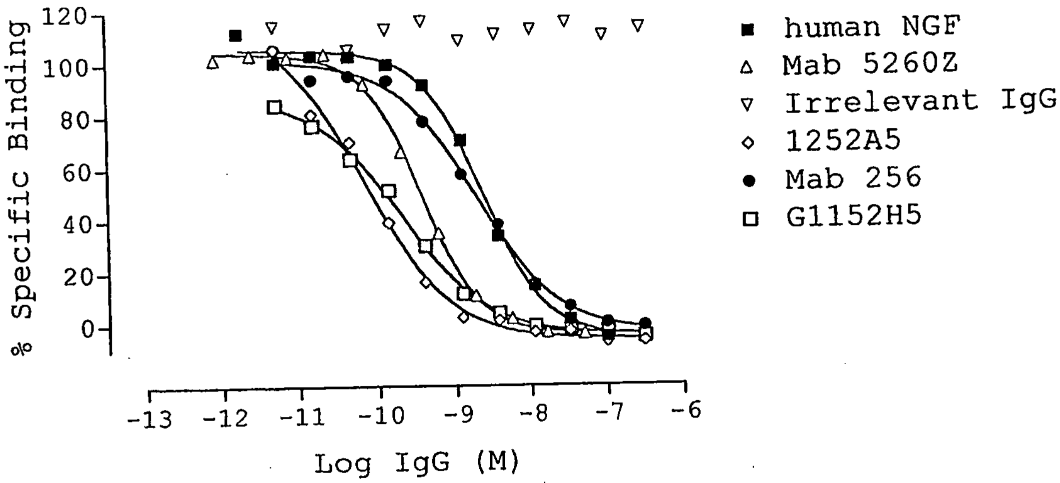


Figure 8B

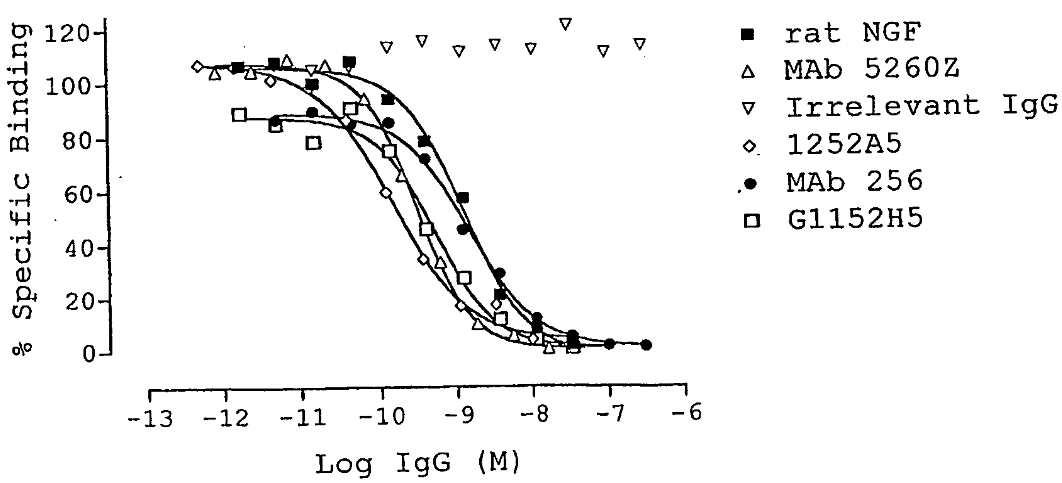


Figure 9A

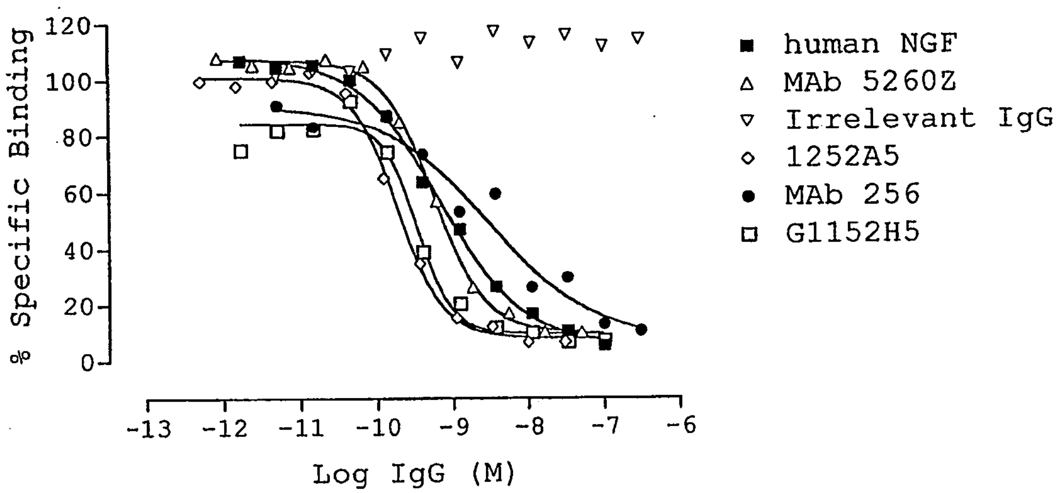


Figure 9B

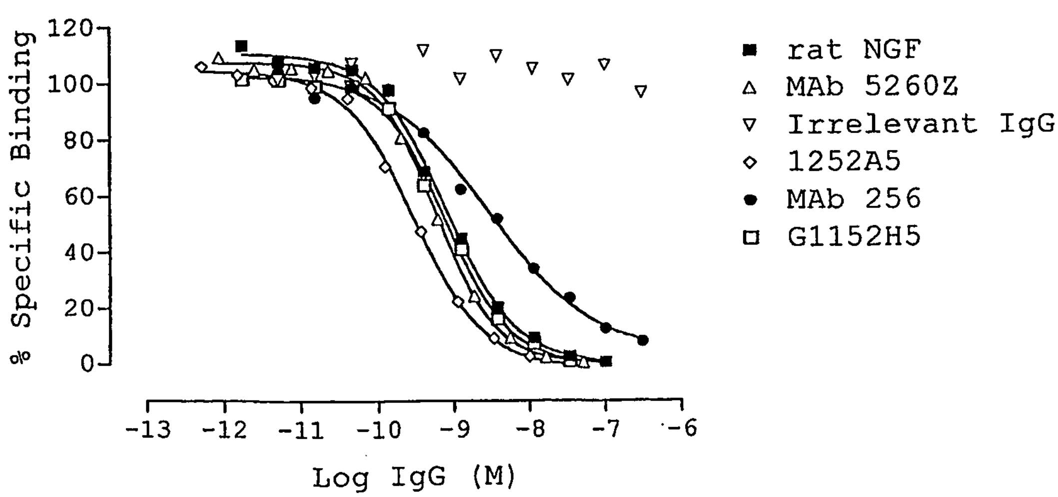
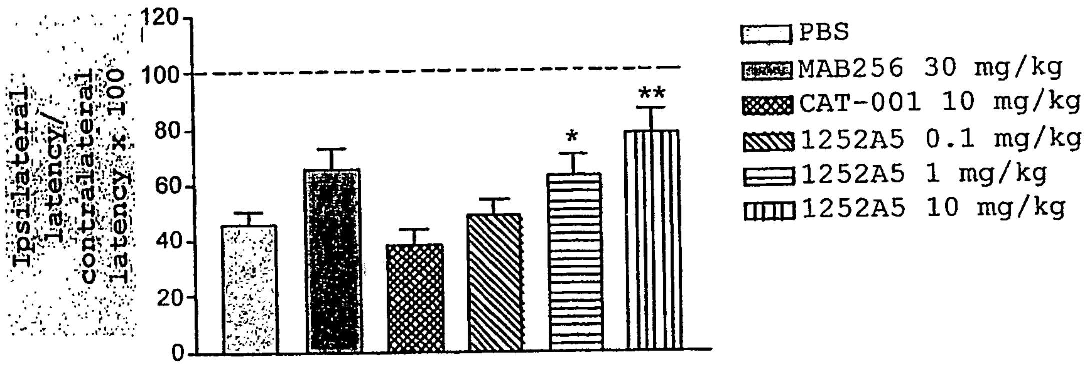


FIGURE 10



*P<0.05 and **P<0.01 c.f. CAT-001 null isotype antibody control (n=13-16 animals per group)

SEQUENCE LISTING

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<110> Elan Pharma International Limited

<120> Specific Binding Members for NGF

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gcacagaagt tccagggcag actcacgatt accgcggacg aattcacgag cacagcccat     240

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Ser Val Lys Val Ser Cys Lys Ala Ser Gly Gly Thr Phe Ser Ser Tyr
            20                  25                  30
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INTERNATIONAL SEARCH REPORT

International application No
PCT/GB2006/000238

A. CLASSIFICATION OF SUBJECT MATTER
INV. C07K16/22 C07K14/48

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
C07K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

EPO-Internal, WPI Data, BIOSIS, Sequence Search

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WARREN S L ET AL: "Inhibition of biological activity of mouse beta-nerve growth factor by monoclonal antibody." SCIENCE. 21 NOV 1980, vol. 210, no. 4472, 21 November 1980 (1980-11-21), pages 910-912, XP009064834 ISSN: 0036-8075 abstract	1-72
X	WO 02/096458 A (GENENTECH, INC; SHELTON, DAVID, L) 5 December 2002 (2002-12-05) abstract; claims 1-47	1-72
X	WO 00/73344 A (SIRS SOCIETA' ITALIANA PER LA RICERCA SCIENTIFICA; NOVAK, MICHAL, M; L) 7 December 2000 (2000-12-07) abstract; claims 1-24	1-72

-/--

[X] Further documents are listed in the continuation of Box C.

[X] See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
11 April 2006	11/05/2006

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL – 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Favre, N

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 92/09631 A (ABBOTT LABORATORIES) 11 June 1992 (1992-06-11) abstract; claims 1-16	1-72
X	OKISHIO MIKI ET AL: "Establishment of monoclonal antibodies against human nerve growth factor" BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS, vol. 196, no. 3, 1993, pages 1474-1480, XP002376627 ISSN: 0006-291X abstract	1-72
X	RO LONG-SUN ET AL: "Effect of NGF and anti-NGF on neuropathic pain in rats following chronic constriction injury of the sciatic nerve" PAIN, vol. 79, no. 2-3, February 1999 (1999-02), pages 265-274, XP002376628 ISSN: 0304-3959 abstract	1-72
X	WO 01/44300 A (CAMBRIDGE ANTIBODY TECHNOLOGY LIMITED; WEBSTER, CARL; OSBOURN, JANE; W) 21 June 2001 (2001-06-21) sequence 48	28-36
X	WO 2004/050683 A (ABGENIX, INC; BABCOOK, JOHN, S; KANG, JASPAL, S; FOORD, ORIT; GREEN, L) 17 June 2004 (2004-06-17) sequence 309	28-36
X	WO 01/27279 A (CAMBRIDGE ANTIBODY TECHNOLOGY; EDWARDS, BRYAN, MICHAEL; MAIN, SARAH, H) 19 April 2001 (2001-04-19) sequence 84	28-36
X	WO 03/030833 A (AMGEN INC; OLINER, JOHNATHAN, DANIEL) 17 April 2003 (2003-04-17) sequence 35	28-36
X	WO 03/102136 A (HUMAN GENOME SCIENCES, INC; ROSEN, CRAIG, A; RUBEN, STEVEN, M) 11 December 2003 (2003-12-11) sequence 37	28-36

INTERNATIONAL SEARCH REPORT

Box II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

Although claims 70-72 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

2. [ ] Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/GB2006/000238

Patent document cited in search report		Publication date	Patent family member(s)			Publication date
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			JP	6503722	T	28-04-1994
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			CA	2393292	A1	21-06-2001
			EP	1242458	A2	25-09-2002
			JP	2003516745	T	20-05-2003
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			JP	2005506067	T	03-03-2005
			MX	PA04003345	A	08-07-2004
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